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Antimicrobial active packaging with biopolymers and natural extracts: sustainable solutions and technological challenges

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The growing demand for sustainable food packaging solutions has increased research into antimicrobial active packaging systems based on biodegradable biopolymers and natural extracts. This review provides a comprehensive overview of recent progress in their development, functionality, and existing challenges, with particular emphasis on plant-derived compounds, animal-derived antimicrobials, and microbial metabolites. These bioactive agents exhibit various antimicrobial mechanisms, including membrane disruption, enzyme inhibition, and oxidative stress induction, and are often incorporated into biopolymer matrices through solvent casting, extrusion, or encapsulation techniques. However, the critical technological challenge lies in achieving the stability, compatibility, and controlled release of these agents in complex food matrices, which directly impacts their real-world applicability. Regulatory restrictions, economic feasibility, and consumer safety concerns further complicate commercialization. By synthesizing current findings, this review emphasizes that emerging encapsulation strategies and smart delivery systems not only enhance antimicrobial efficacy but also represent a pivotal step toward scalable, eco-friendly, and truly sustainable packaging solutions. Therefore, this review highlights how emerging technologies and policy approaches can integrate material innovation with sustainability goals to create safer, more effective, and eco-friendly antimicrobial packaging.

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Sustainability spotlight

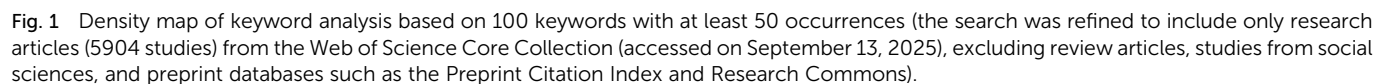
This review highlights the potential of biopolymer- and natural extract-based antimicrobial active packaging to reduce food spoilage, extend shelf-life, and minimize reliance on petroleum-based plastics. The advancements in their fabrication and application directly support global sustainability goals, including SDG 12 (Responsible Consumption and Production) and SDG 14 (Life Below Water), by addressing both food waste and plastic pollution. This article also emphasizes the importance of encapsulation and controlled-release technologies to increase the stability and efficacy of natural antimicrobial agents, paving the way for scalable, safe, and multifunctional packaging solutions. Through a comprehensive synthesis of recent advances, regulatory frameworks, and engineering strategies, this work contributes to the development of sustainable food preservation technologies that align with consumer expectations, environmental mandates, and circular economy principles.

1. Introduction

Research on food packaging is evolving, from containment to now being active, intelligent, and smart. Petroleum-based packaging continues to dominate owing to its superior protective properties, but its persistence in the environment has resulted in serious ecological and health concerns, including the spread of microplastics that now affect both humans and other living organisms. According to the World Health Organization,¹ health risks occur throughout the plastic lifecycle, from production to disposal, whereas global initiatives such as the Sustainable Development Goals (SDGs), particularly SDG 12 (Responsible Consumption and Production) and SDG 14 (Life Below Water), emphasize the need for sustainable packaging and reduced plastic pollution.² In this context, biopolymers are

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Therefore, this review aims to explore the recent progress in antimicrobial active packaging based on biopolymers and natural extracts, with a focus on studies published after 2015. It critically examines how plant-derived (essential oils, flavonoids, polyphenols, tannins), animal-derived (lysozyme, lactoferrin, chitosan derivatives), and microbial (nisin, bacteriocins, peptides) antimicrobial components interact with biopolymer matrices to extend shelf-life and enhance food safety. Particular



attention is given to the mechanisms governing antimicrobial action, release behavior, and material performance, as well as to emerging engineering strategies such as encapsulation, nanostructuring, and advanced coatings that improve stability and efficacy. Moreover, the review highlights associated challenges, such as compound instability and matrix compatibility, as well as safety and regulatory challenges. By highlighting these sustainability trade-offs and integrating advanced engineering strategies, the present review establishes antimicrobial biopolymer packaging as an important step toward more sustainable ways to preserve food.

2. Natural antimicrobial extract

The active ingredients within the food packaging system play a significant role in food quality. It enhances the shelf-life of food by releasing desirable components that inactivate microbes. In addition, bioactive compounds from natural extracts are strongly compatible with biopolymers and often produce synergistic effects, enhancing antimicrobial activity through the controlled release of active compounds. The mechanisms involved in reducing a wide range of microbes using natural extracts are discussed in further sections. Moreover, Table 1 presents a list of some common antimicrobial components in natural extracts, their sources and their mechanism of action.

2.1 Plant derivative extract

Plant-derived extracts such as essential oils, polyphenols, and phytochemicals are widely incorporated into packaging material matrices because of their effective bioactive properties. For example, essential oils are volatile aromatic secondary metabolites that are extracted from various parts of angiospermic plant tissues and serve ecological functions in pollination and defense against microbial pathogens.¹³ Similarly, essential oils are compatible with biopolymer-based packaging in the form of films and coatings. When mixed with a biopolymer solution, they form a stable emulsion that, upon drying, becomes embedded in the polymer matrix, providing antimicrobial activity through the migration of active compounds from the matrix to the food. Scanning electron microscopy (SEM) images confirm that the essential oils cause structural alterations in the film during drying, which results from the complex interactions that take place between the lipid, the protein and the solvent. These phenomena increase the stability of the essential oils in the matrix. The bioavailability of these active compounds is directly correlated with the concentration of the essential oils being incorporated and the temperature of the system.²⁹ Furthermore, its molecular weight is less than 300 Da, which results in high vapor pressure at room temperature, allowing it to exist in a gaseous state.³⁰ The antimicrobial activity of essential oils is directly associated with the presence of bioactive volatile chemical compounds, primarily monoterpenes, sesquiterpene hydrocarbons and their oxygenated derivatives.³¹ The mechanism of action of essential oils against microbes involves disruption of bacterial cellular membranes, which

leads to increased permeability and leakage of intracellular contents. This is due to the accumulation of bioactive components of the essential oils, which are adsorbed onto the cell surface and subsequently penetrate the phospholipid bilayer of the membrane. Alterations in membrane permeability result in the leakage of essential intercellular components, including proteins, reducing sugars, ATP, and DNA. It simultaneously disrupts ATP generation and associated enzymatic activities, ultimately leading to cellular destruction and electrolyte loss.³² Moreover, it also affects bacterial dissipation of the proton motive force and adenosine triphosphate depletion. Even prolonged exposure to essential oils triggers apoptosis and necrosis in bacterial cells, disrupts ion transport *via* interactions with membrane proteins and intercellular compounds, impairs enzyme activity, and causes electrolyte loss (K^+ , Na^+ , Ca^{2+}).³⁰ These effects arise through multiple pathways and are influenced by their hydrophobic and hydrophilic nature, bacterial type, and cell wall structure. For example, Gram-positive bacteria are particularly susceptible to essential oils, as their peptidoglycan cell wall allows hydrophobic compounds (mostly present in essential oils) to penetrate and interact with the cytoplasm. However, in a few cases, Gram-negative bacteria resist infection *via* an additional outer membrane consisting of proteins and lipopolysaccharides around the cell wall.³³

On the other hand, polyphenols such as flavonoids, flavan-3-ols, flavonols, flavanones, phenolic acids, stilbenoids, and tannins are nonvolatile plant secondary metabolites with multiple phenol rings and are incorporated in active packing material matrices because of their antimicrobial properties.³⁴ Sachets, absorbent pads, biopolymer coatings, *etc.*, are commonly used as vehicles for polyphenol-based antimicrobial packaging materials.³⁴ The incorporation and stability are accomplished using intermolecular bonding *via* hydrophobic forces and electrostatic interactions between the functional groups of the packaging material matrix and polyphenols. Furthermore, antimicrobial action occurs by contact diffusion of active compounds of polyphenols from films or coating into food.³⁵ The bioavailability of these active compounds is directly correlated with the amount of fat and moisture present in the food matrix.²⁹ These polyphenols inhibit bacterial biofilm formation, a protective layer against adverse factors, through mechanisms such as reducing motility and surface adhesion, blocking detection, and inhibiting the expression of virulence factors associated with pathogenic behavior.^{36,37} The inhibition of biofilm formation can be achieved by modifying the activity of curli genes (*csgA*, *csgB*) in *E. coli* O157:H7, increasing the level of protein IsaA (immunodominant staphylococcal antigen A) for *S. aureus* and the formation of hydrogen bonds *via* hydroxyl groups.³⁴ Additionally, polyphenols disrupt the cell membranes of Gram-positive and Gram-negative bacteria by altering their phospholipid or lipid bilayers, resulting in reduced fluidity, increased permeability, and impaired ion transportation.³⁸ It binds with the peptidoglycan of the cell wall, thereby lowering osmotic pressure and increasing ionic strength, which reduces cell tolerance.³⁹ Moreover, it leaks small molecules from the liposome inner space to agglomerate the liposome, causing membrane lipid bilayer damage and





Table 1 Antimicrobial components, their natural sources and their mechanism of action against microbes

Antimicrobial components	Sources	Mechanism of action	Optimal working factors	References
Cinnamaldehyde	Cinnamon essential oil	PMK-1 mediated p38 signaling pathway against <i>P. aeruginosa</i>	MIC of 10 mg L ⁻¹ and 100 mg L ⁻¹	Shu <i>et al.</i> ¹⁴
Eugenol	Clove essential oil	Alters membrane permeability, resulting in leakage of intracellular contents against <i>E. coli</i>	MIC of 0.0312 to 8 µg mL ⁻¹ within 4 h	Jeyakumar & Lawrence ¹⁵
1,8-Cineole and α-pinene	Rosemary essential oil	Cell wall stress, destruction of organelle membranes, vacuolar segregation, mitochondrial depolarization, and cell cycle arrest at G1/S phase against <i>Candida albicans</i>	MIC of 4500 µg mL ⁻¹ for 1,8-cineole and 3125 µg mL ⁻¹ for α-pinene	Shahina <i>et al.</i> ¹⁶
Carvacrol	Lamiaceae essential oil	<i>B. subtilis</i> 168 elongation and <i>S. aureus</i> BAA-41 enlargement and inhibition of cell division	MIC of 4–16 µg mL ⁻¹	Zhi <i>et al.</i> ¹⁷
Citral	Citrus essential oils	Decreased intracellular ATP concentration, and cell membrane hyperpolarization against <i>Cronobacter sakazakii</i>	MIC of 0.27 to 0.54 mg mL ⁻¹	Shi <i>et al.</i> ¹⁸
Chrysoeriol-7-O-β-D-xyloside, luteolin-7-O-β-D-apiofuranosyl-1 → 2-β-D-xylopyranoside, chrysoeriol-7-O-β-D-apiofuranosyl-1 → 2-β-D-xylopyranoside, chrysoeriol-7-O-α-L-rhamnopyranosyl-1 → 6-β-D-4'-hydrogeno sulfate glucopyranoside, and isorhamnetin-3-O-α-L-rhamnopyranosyl-1 → 6-β-D-glucopyranoside	<i>G. glandulosum</i> Flavonoids glycosides	Nucleic acids lost through a damaged cytoplasmic membrane against <i>V. cholerae</i>	MIC value of 64 µg mL ⁻¹ for was recorded with compound chrysoeriol-7-O-α-L-rhamnopyranosyl-1 → 6-β-D-4'-hydrogeno sulfate glucopyranoside	Tagousop <i>et al.</i> ¹⁹
Pentagalloyl-O-β-D-glucose	Cytinus tannin extracts	Destruction of membranes and chelation of metal ions against <i>Staphylococcus aureus</i> , <i>Staphylococcus epidermidis</i> , <i>Enterococcus faecium</i>	MIC of 125 to 500 µg mL ⁻¹	Maisetta <i>et al.</i> ²⁰
1,5-Di-O-caffeoyl quinic acid	<i>L. crithmoides</i> phenolic extracts	Destruction of cell wall against <i>Staphylococcus aureus</i> ATCC 25923, and <i>Pseudomonas aeruginosa</i> ATCC 27853, <i>Escherichia coli</i> ATCC 3521, <i>Enterococcus faecalis</i> ATCC 29212, and <i>Salmonellatyphimurium</i> LT2 DT104	ZI > 1–16 mm	Jallali <i>et al.</i> ²¹
Oleiferasaponin D ₃	<i>C. oleifera</i> cake saponin extract	Destruction of cell membrane structure, leakage of cell contents and inhibited the growth of mycelium, reduced cell adhesion and aggregation, and effectively inhibited the formation of biofilm against <i>C. albicans</i> , <i>S. cerevisiae</i> , and <i>Penicillium</i>	MIC of 0.078, 0.156, and 0.156 mg mL ⁻¹	Yu <i>et al.</i> ²²
Amyloids fibrils, peptide residues, and worms	Hen egg white lysozyme	Electrostatic binding to the cell walls and membranes of the microorganisms, followed by membrane disruption against <i>S. aureus</i> and <i>C. albicans</i>	MIC of 0.01 mg mL ⁻¹	Kummer <i>et al.</i> ²³

Table 1 (Contd.)

Antimicrobial components	Sources	Mechanism of action	Optimal working factors	References
Lactoferricin Lfcin and lactoferrampin Lfampin	Red deer <i>Cervus elaphus</i> milk lactoferrin	Form amphipathic structures with net hydrophobic and positively charged surfaces against <i>E. coli</i> ATCC 25922 and <i>L. acidophilus</i> ATCC 4356	MIC of 240–480 $\mu\text{g mL}^{-1}$	Wang <i>et al.</i> ²⁴
Lactoperoxidase, glucose oxidase, α -D-glucose, potassium thiocyanate, and hydrogen peroxide	Lactoperoxidase system	Oxidation of essential sulphhydryl groups in bacterial enzymes and proteins against psychrotrophic bacteria and <i>Pseudomonas</i> spp.	Concentration of 5–7.5%	Farshidi <i>et al.</i> ²⁵
Thiazolium moieties, quaternized 4-2-4-methylthiazol-5-yl ethoxy-4-oxobutanoic acid	Chitosan from shrimp shells	Permeabilize the cell wall against <i>L. innocua</i> , <i>C. albicans</i> , <i>S. epidermidis</i> , <i>S. aureus</i> , <i>E. coli</i>	MIC of 8–32 $\mu\text{g mL}^{-1}$	Muñoz-Núñez <i>et al.</i> ²⁶
Nisin	<i>Lactobacillus</i> sp.	Forming pores and inhibiting cell wall synthesis against <i>E. faecalis</i> strains ATCC 29212, OGIRF, and strain E	MICs of 1 mg mL^{-1}	Tong <i>et al.</i> ²⁷
Reuterin	<i>Lactobacillus reuteri</i>	Targets lipid and amino acid metabolism, leading to cell membrane damage, subsequently results in energy metabolism disorder against <i>Staphylococcus aureus</i>	MIC of 18.25 mM	Sun <i>et al.</i> ²⁸

apoptosis.⁴⁰ The B rings of flavonoids inhibit DNA and RNA synthesis by intercalating or hydrogen bonding with nucleic acid bases, while they also block nucleic acid synthesis by inhibiting DNA gyrase.⁴¹ In addition, polyphenols bind proteins both noncovalently and covalently, exerting antibacterial activities by sequestration and denaturation of proteins into soluble or insoluble complexes.³⁴

In addition to polyphenols and essential oils, several other compounds in plant-derived extracts exhibit strong antimicrobial activity. Alkaloids, terpenoids, lectins, quinone, coumarin, polypeptides, tannins (nonphenolic), and sulfide-containing compounds interfere with microbial DNA replication, RNA transcription, and protein synthesis; inhibit biofilms, cell division, and enzyme activity; and disrupt the cell wall.^{42–45} In conclusion, plant-derived extracts exhibit strong antimicrobial potential through various mechanisms. However, their application can be limited by factors such as high volatility, which affects the organoleptic properties of food due to their strong aroma, poor water solubility, heat and light sensitivity, and regulatory and safety considerations.²⁹ In addition, the incorporation of these extracts reduced the film strength and increased the water vapor permeability.⁴⁶ The possible accumulation and uneven distribution of these extracts on the surface of the film and deterioration of its properties (*e.g.*, oxidation, thermal degradation) were also observed.⁴⁷ Therefore, advanced stabilization and controlled-release strategies such as encapsulation or nanodelivery systems are commonly employed, as discussed later.

2.2 Animal derivative extract

Numerous animal-derived biopolymers, enzymes and proteins, such as lysozyme, lactoferrin, chitosan derivatives, and lactoperoxidase, have been effectively leveraged in active packaging materials to inhibit microbial growth. Lysozyme (*N*-acetylmuramic hydrolase) is a cysteine-rich disulfide-linked hydrolytic enzyme of animal origin (found in egg and milk) that is generally recognized as safe (GRAS) and is widely recognized for its antimicrobial activity.⁴³ Lysozymes exhibit excellent stability and solubility in water, making them blend effectively with a film-forming solution. Therefore, it can be used as an edible film, coating, or applied directly to the surface of foods. To apply a coating, the food product is immersed in the film-forming solution and subsequently dried, resulting in the formation of a microfilm on the food surface. The bioavailability of lysozyme was highest with chitosan. It is evenly distributed in chitosan-based biopolymer films through hydrogen bonds (N–H in lysozyme and O–H in chitosan) and shows high antimicrobial activity *via* pH-dependent controlled release.⁴⁸ During storage, lysozyme hydrolyzes chitosan to smaller molecular weights with higher antimicrobial activity while retaining its potential as an antimicrobial agent.⁴⁹ Several factors, such as pH, temperature, ionic strength, and salt type, significantly influence lysozyme bioavailability. It exhibits optimum activity at pH 5, while its stability decreases under highly acidic and alkaline conditions. Low concentrations of salts normally increase the catalytic efficiency of lysozyme, but

high ionic strengths inhibit its activity. Lysozyme is a thermally stable enzyme. It remains active even at high temperatures, and its activity can be further protected by sugars and polyols.^{50,51} Lysozyme cleaves the β -(1,4)-glycosidic linkage between *N*-acetylmuramic acid and *N*-acetylglucosamine in peptidoglycan chains, a key structural element of the cell wall of Gram-positive bacteria, and this hydrolytic activity weakens wall integrity, ultimately leading to bacterial lysis. However, they are ineffective against Gram-negative bacteria because their outer membrane is rich in phospholipids, lipopolysaccharides, and lipoproteins that block access to peptidoglycan. However, in some cases, as a cationic protein, lysozyme can still form pores in bacterial cells *via* electrostatic interactions with membrane phospholipids, causing lysis independent of peptidoglycan hydrolysis.⁵² The cationic, hydrophilic, and lipophilic properties of lysozyme increase its affinity for the negatively charged outer membrane of bacteria, subsequently leading to disruption of the integrity of the plasma membrane.⁵³ Therefore, lysozyme can be incorporated into packing matrices to develop effective antimicrobial packaging systems.

On the other hand, lactoferrin is an iron-binding glycoprotein present in milk that has antimicrobial effects on Gram-positive and Gram-negative bacteria, viruses, and fungi through iron deprivation. It interacts with bacterial membranes through its lactoferricin peptide.⁵⁴ Its function depends on its ability to bind iron, which results in iron deficiency, restricting bacterial growth.⁴³ Lactoferrins are compatible with biopolymer films or coatings such as gelatin, chitosan, cellulose, and nanocellulose *via* electrostatic interactions. They also exhibit antimicrobial activity through direct contact migration and controlled release.⁵⁵ Furthermore, the lactoperoxidase system (LPOS), which is naturally present in milk and human saliva, comprises three essential components, namely, lactoperoxidase (LPO), hydrogen peroxide (H_2O_2), and thiocyanate (SCN^-), and only displays antimicrobial effects when they coexist. They are mostly bioavailable with chitosan-, whey protein- and alginate-based biopolymer films and are stable *via* hydrogen bonding or charge-charge interactions.⁴⁸ These films show antimicrobial activity through the controlled diffusion of active agents to food surfaces.⁵⁶ Within this system, LPO catalyzes the oxidation of SCN^- to generate hypothiocytosis (OSCN^-) and hypothiocytosis acid (HOSCN), which are reactive species that oxidize sulfhydryl ($-\text{SH}$) groups in microbial proteins and enzymes, thereby disrupting cellular metabolism.⁵⁶ The oxidation of the SH group damaged the cell wall structure. This damage makes the wall more permeable, allowing important nutrients and cell components, such as ions, amino acids, and peptides, to leak out. This can hinder the growth of microorganisms and may lead to cell death. By this mechanism, LPOS exhibits broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria, along with antifungal effects, making it effective for antimicrobial packing applications.

In addition to these proteins and enzymes, chitosan and its derivatives, polysaccharides obtained from crustacean shells, have also gained prominence as animal-derived biopolymer films in antimicrobial packaging. Chitosan-based polymeric materials can be formed into fibers, films, gels, sponges, beads

or nanoparticles. It exerts its activity despite its cationic amino groups, which interact electrostatically with negatively charged microbial cell membranes, leading to increased permeability, leakage of intercellular components, and eventual cell death. The bioavailability of chitosan in terms of its antimicrobial effect on a packaging material significantly depends upon several intrinsic and extrinsic factors, such as concentration, pH, molecular weight, and solubility. Chitosan has a concentration-dependent antimicrobial effect. Similarly, at low concentrations, its protonated amino groups bind to negatively charged bacterial surfaces, disrupt membrane integrity, and promote leakage of intercellular components, whereas at higher concentrations, chitosan forms a uniform coating on the cell surface, which suppresses further leakage. The optimal condition is 1–5% chitosan (w/w) with LDPE for packaging applications.^{57,58} Low-molecular-weight (<4.6 kDa) chitosan can also enter the cell's nucleus and block the transcription of RNA from DNA through adsorption to DNA molecules,⁵⁹ exerting antimicrobial effects on bacteria, yeasts, and fungi.⁶⁰ Furthermore, at a relatively high charge density of 83.7%, strong electrostatic interactions occur, leading to an outstanding antimicrobial effect.⁶¹ The hydrophilic–hydrophobic balance of chitosan is another important factor determining its antimicrobial activity. The poor water solubility of chitosan creates major restrictions. However, hydrophobic modifications, including *N*-acylation, improve the affinity of this polymer for microbial membranes. Chitosan becomes highly soluble and polycationic in acids. Therefore, its antimicrobial activity is far stronger at low pH and decreases close to neutral. Moreover, it has a high chelating ability toward metal ions such as Zn^{2+} , Fe^{2+} , and Mg^{2+} at low pH, showing antimicrobial effects by destabilizing microbial cell walls through the sequestration of essential ions.^{57,60,62}

Furthermore, saturated fatty acids (SAFAs), mono-unsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) extracted from *Salmo salar* fish waste oil showed antimicrobial activity against Gram-positive and Gram-negative bacteria. The extracted fatty acids alter bacterial cell membrane hydrophobicity, charge and integrity, which results in electron leakage and subsequent cell death.⁶³ Similarly, α -helical pardaxins isolated from the skin glands of Red Sea Moses sole (*Pardachirus marmoratus*) show antimicrobial activity against Gram-positive and Gram-negative bacteria *via* pore-forming activities. Additionally, α -helical peptides (pleurocidins and piscidins) from the skin mucus of winter flounder (*Pleuronectes americanus*) inhibit bacterial DNA, RNA, and protein synthesis.⁶⁴ Overall, animal-derived extracts show strong antimicrobial potential, highlighting their value in active packaging. However, their sensitivity to processing and low compatibility limit their commercial use. For example, lactoferrin is highly unstable under certain conditions, such as high temperature and low pH, which cause conformational changes and loss of activity.⁵⁵ On the other hand, the compatibility of LPOS with biopolymers is limited, as it is effectively restricted to alginate, chitosan, gelatin, and whey protein. Moreover, it depends on a continuous or sequential cofactor supply, making its direct incorporation into packaging films difficult.⁵⁶ Overall, compared with the plant-derived extract, neither group was



universally superior. Each performs best under specific food and packaging conditions, and their optimal choice depends on the desired release behavior, target microorganisms, and product formulation. Moreover, combining them with plant-based extracts may offer synergistic, sustainable, and multi-functional food packaging solutions. Therefore, more research should focus on immobilization, encapsulation, and compatibility with a wide range of biopolymers, as well as synergistic and comparative studies with other natural extracts, to improve their use in real-world food packaging applications.

2.3 Microbial derivative extract

Antimicrobial compounds extracted from bacterial cells, known as bacteriocins, are gaining potential for use in packaging material matrices because of their ability to resist microbes and tolerate high temperatures and acidic environments. These are metabolic byproducts that bacteria produce as part of their defense mechanism. The bacteriocins produced by lactic acid bacteria, known as nisin, are acceptable because of their traditional role in the food fermentation process. Nisin belongs to the lantibiotic class of bacteriocins and is composed of 34 amino acids. It interacts with the bacterial cell wall, forming pores that disrupt the pH equilibrium and proton motive force, causing cellular fluid leakage and cell death. Nisin is more effective against spores than it is against vegetative microorganisms because of its sporostatic potential. It interacts with biopolymers through a polymer network during film preparation, becoming stable and exhibiting antimicrobial activity through pH-dependent diffusion from the biopolymer matrix to the microbial surface. The compatibility and bioavailability of nisin with the packaging material matrix are excellent, with a polyvinyl dichloride lacquer coating ($800 \pm 7 \text{ IU dm}^{-2}$), chitosan films ($1.234\text{--}1.347 \times 10^{-13} \text{ m}^2 \text{ s}^{-1}$), and composite films (0.6–2.0 wt% of total released). During nisin release from food packaging, water first diffuses into the material matrix, causing structural relaxation and swelling, which enables the antimicrobial agent to migrate through the matrix and ultimately reach and act upon the microbial surface.^{65–67}

On the other hand, pediocin forms a random coil in aqueous solution, which becomes partially helical with distinct hydrophobicity in nonaqueous media. Its N-terminal cationic and C-terminal hydrophobic regions enable pore formation in bacterial membranes, causing leakage of cellular fluids, ATP loss, proton motive force collapse, and cell death.⁶⁸ The polylactic acid–sawdust particle biocomposite film surface was impregnated with pediocin by diffusion coating, which resulted in an antimicrobial effect. The sawdust particles increased the surface hydrophilicity, which facilitated the adsorption and controlled release of hydrophilic pediocin to the surface of the bacteria.⁶⁹ Similarly, reuterin (β hydroxypropionaldehyde), produced from glycerol by certain strains of *Lactobacillus reuteri*, is another antimicrobial agent attracting interest because of its high water solubility, resistance to heat, proteolytic and lipolytic enzymes, and stability over a wide range of pH values. Alginate–konjac gum film containing *L. reuteri* cells can produce reuterin through anaerobic glycerol bioconversion,

resulting in antimicrobial activity.⁷⁰ The aldehyde functional group in reuterin acts as a bioactive component, which triggers oxidative stress in microbes by altering thiol groups in proteins and small molecules.⁷¹ An alternative mechanism suggests that the dimeric form of reuterin interferes with DNA synthesis, thereby suppressing microbial growth and causing cell death.⁷² In addition to microbe-derived biochemical extracts, bacteriophages may serve as effective antimicrobial agents within active packaging materials composed of acetate cellulose. The incorporation of bacteriophage into the cellulose film demonstrated observable diffusion into the bacterial medium through contact with the inoculated environment.⁷³ Bacteriophages encode enzymes that effectively hydrolyze the cell wall peptidoglycan core and lyse bacterial host cells during the terminal phase of their lytic cycle.⁷⁴ All these microbiological derivatives highlight their potential for use as antimicrobial packing agents. While their natural origin, stability and broad antimicrobial mechanism make them attractive, challenges are present. For example, direct incorporation of pediocin in the packaging material matrix faces notable challenges, as it can be rapidly degraded by proteolytic enzymes and adsorbed by the food matrix, leading to diminished activity during long-term storage or under inadequate temperature conditions.⁶⁸ Similarly, when unencapsulated nisin is incorporated into a packaging material matrix, it quickly loses its activity through degradation due to environmental factors such as temperature, pH, oxygen, *etc.*⁶⁶ However, in comparison with plant and animal extracts, microbial metabolites demonstrate greater reliability in controlled-release packaging systems.

Moreover, active packaging systems with natural extracts often lack the ability to distinguish between harmful pathogens and beneficial or neutral microbiota. Therefore, future research should prioritize the development of smart-responsive packaging, genetic engineering of microbes, phage–biofilm synergy, and hybrid nanostructures for embedding these compounds. Future research should also be conducted in the design and optimization of engineered antimicrobial packaging to target pathogens while preserving or leaving beneficial or neutral microbiota. Variations in membrane charge, peptidoglycan architecture, and lipid composition in many pathogens make them inherently more susceptible to the action of mechanisms that disrupt membranes. Moreover, few bioactive agents, such as lysozyme or nisin, act upon specific targets, which leads to stronger effects on Gram-positive or spore-forming organisms. Moreover, the MICs and release rates further guide the formulations to maintain concentrations that impede pathogens while nontarget microbes are unaffected.

2.4 Encapsulation methods for natural extracts

Natural extracts from plants, animals, and microorganisms face challenges such as environmental sensitivity and instability. Several encapsulation techniques, such as solvent evaporation, phase inversion, spray drying, freeze drying, cross-linking, and ionic gelation, might be beneficial, as they preserve the effectiveness of these natural extracts and improve target-specific delivery, oxidation stability, and solubility. The solvent



evaporation method is a widely used low-energy encapsulation method that involves dissolving the natural extract in a polymer-organic solvent, emulsifying it in an aqueous phase, and solidifying it as the solvent evaporates, forming polymeric microspheres. Natural extracts from plants are primarily encapsulated using this method.⁷⁵ Another low-energy encapsulation method is phase inversion. In this method, changes in temperature or composition alter the surfactant affinity, which leads to the formation of fine oil-in-water emulsions. This material is effectively used to encapsulate bioactive compounds such as quercetin, epigallocatechin-3-gallate, flavonoids, and vitamin E.⁷⁶

In contrast, encapsulation using the spray-drying method results in the formation of a protective shell around the active core ingredients. Natural or synthetic polymers are used for this purpose, with protein- and polysaccharide-based biopolymers being the most common owing to their biocompatibility and biodegradability. This technique has been effectively applied to encapsulate a wide range of bioactive compounds, including plant-derived substances such as essential oils and polyphenols;^{77,78} animal-derived components such as lysozyme,⁷⁹ lactoferrin,⁸⁰ and lactoperoxidase;⁸¹ and bacterial derivatives such as bacteriocins.⁸² Natural extracts such as polyphenols, lysozyme, and bacteriocins can also be encapsulated using cross-linking methods. In this method, biopolymers, *e.g.*, proteins and polysaccharides, are chemically cross-linked using various agents, resulting in a three-dimensional network that entraps these bioactive compounds.^{83–85}

Freeze drying, on the other hand, is suitable for heat-sensitive plant bioactive compounds; bacterial extract encapsulated as the feed emulsion is frozen at subzero temperatures, and the ice crystals are sublimated under low pressure.⁷⁵ Additionally, for heat-sensitive hydrophobic compounds, the encapsulation method is ionic gelation, where a polymeric solution with an active compound is atomized into an ionic solution, forming spherical gel structures. This simple and cost-effective method does not require special equipment.⁸⁶

Nanotechnology-enabled encapsulation enhances the stability, bioavailability, and controlled release of natural extracts by incorporating them into nanoscale carriers such as liposomes, nano emulsions, and nanoparticles. Liposomes are spherical vesicles that range in size from 30 nanometers to the micrometer scale. They are composed of phospholipids that spontaneously arrange themselves into lipid bilayers. Liposomes are used to encapsulate and deliver essential oils such as *Zanthoxylum tingoassuiba* and Laurel,⁸⁷ as well as polyphenols such as curcumin, resveratrol, quercetin, honokiol, and anthocyanins.⁸⁸ Additionally, liposomes can be loaded with β -carotene through co-modification with chitosan and lactoferrin.⁸⁹ On the other hand, nano emulsions are conventional emulsions with droplet sizes smaller than 200 nm. While they are thermodynamically unstable, their small size offers long-term stability, enhanced bioavailability, and improved transparency. They are widely used to encapsulate essential oils, polyphenols, lysozyme, and bacteriocins.^{90–93} Studies have shown that nisin can be loaded with mesoporous silica

nanoparticles, poly- γ -glutamic acid/poly-L-lysine nanoparticles, and silver nanoparticles.⁶⁶

However, the shelf-life and stability of these encapsulated natural extracts may change under different storage conditions. For example, temperature has a role in the oxidative stability of encapsulated essential oils during storage. Oxidative degradation was lower in Citrus reticulata essential oil stored at 5 °C than in that stored at 50 °C for 30 days.⁹⁴ Similarly, the oxidative stability of encapsulated *P. jaubertii* extract was greater at 4 °C than at 25 °C.⁹⁵ The activity of chitosan-thyme oil nanocapsules stored at 4 °C was greater than that of those stored at 25 °C for 5 weeks.⁹⁶ On the other hand, moisture is a crucial factor in terms of the storage stability of lysozyme. The accelerated stability at 40 °C and 75% RH showed that crystallized lysozyme retained 95% activity after 20 weeks, whereas the activity of the spray-dried samples decreased to 87%.⁹⁷ Additionally, storage studies of freeze-dried encapsulated bovine lactoferrin powder at 4 °C and 40% RH for nine years revealed that it has antimicrobial activity against *Salmonella enteritidis*.⁹⁸

Among the encapsulation methods, spray drying offers the highest scalability and moderate cost with the balance of good bioactivity; hence, it is commercially suitable. Freeze-drying results in greater preservation of sensitive compounds but is expensive and less scalable, whereas nano emulsion and nanoparticle systems ensure good retention of bioactivity but require complex and expensive processing. In general, all methods have a trade-off between cost and industrial feasibility with respect to bioactive stability; selection depends on the target compound and intended packaging application. Moreover, the major drawbacks of these encapsulation methods include low efficacy, loss of bioactive compounds, instability during storage, and decreased industrial viability. Ionic gelation can lead to uneven gelation, resulting in soft cores. Therefore, future studies should focus on hybrid methods and scalable low-energy processes to enhance industrial applicability.

2.5 Limitations and safety considerations

One of the primary challenges associated with the application of these natural antimicrobial extracts in food packaging materials is the inadequate compatibility between the polymer-based matrix and the bioactive compounds present in the extract. In most cases, these compounds are not properly incorporated into the polymer matrix. Additionally, the irregular migration of these active compounds toward target microorganisms presents further difficulties. Moreover, growing concerns regarding innovative food packaging continue to impede advancements in the development of effective antimicrobial packaging solutions. Regulatory compliance is essential to ensure consumer safety when packaging comes into direct contact with food. In the European Union, natural antimicrobials such as plant extracts and essential oils are listed as flavoring substances (Regulation EC 872/2012), with some approved as food additives (Regulation EC 1333/2008). In accordance with regulation EC 450/2009, any antimicrobial agents utilized in active packaging that are intended to be released into food products must adhere to regulation EC 1333/2008. This stipulation mandates that such



agents be included on the list of approved food additives.⁹⁹ In the United States, the Food and Drug Administration (FDA) regulates active packing under the Federal Food, Drug, and Cosmetic Act (FFDCA), which classifies incorporated antimicrobials as food additives or GRASs and is also governed by Regulation 1282/2011 on active and intelligent materials, amending Regulations 1935/590/EC and 1989/109/EC.⁴³

3. Biopolymer–natural extract synergy

The synergy between biopolymers and natural extracts influences both the structural integrity and functional performance of antimicrobial packaging systems. To provide a better understanding of these dual contributions, material synergy and functional synergy can be distinguished. Material synergy refers to how polymer–extract interactions affect the mechanical strength, barrier behavior, compatibility, and overall film morphology. In contrast, functional synergy focuses on the controlled release and migration behavior of bioactive compounds, which ultimately governs antimicrobial efficacy.

3.1 Types of biopolymers for antimicrobial packaging

Biopolymers used for antimicrobial packaging can be broadly classified into two different categories: natural biopolymers and synthetic biopolymers. Natural biopolymers can be further divided into three major groups: polysaccharides (chitosan, cellulose, and starch), proteins, and polynucleotides. Polysaccharides, including chitosan, cellulose, and starch, are composed of a number of sugar molecules connected by glycoside linkages. Proteins, such as gelatin and casein, consist of amino acid monomers connected by peptide bonds. Polynucleotides, such as DNA and RNA, are formed from nucleotide monomers. In contrast, synthetic biopolymers include materials such as polylactic acid (PLA), polyesters such as polyhydroxyalkanoates (PHA), and polybutylene succinate (PBS). Biopolymers are eco-friendly and cutting-edge packaging

materials derived from renewable sources, including plants, animals, and microbes, and they are increasingly being explored as greener substitutes for traditional petroleum-derived plastics. Polymers can be utilized in their pure form or can be used in the form of biocomposites with antimicrobial agents such as essential oils or different types of nanoparticles. For a better understanding, the typical classification of biopolymers on the basis of their method of production is illustrated in Fig. 2.

3.2 Material synergy

3.2.1 Interaction mechanisms between biopolymers and natural extracts. Proteins and polysaccharides are the basic components used in food formulations, cosmetics, and pharmaceuticals. The interaction behavior significantly affects the organoleptic properties, visual or appearance, textural, and rheological characteristics of food. The biocomposite edible films can be prepared *via* wet and dry methods. During the preparation of films *via* the wet method, the incorporation of natural plant extracts (such as essential oils, phenolics, and terpenoids) into biopolymer matrices introduces numerous functional groups, including hydroxyl, amino, and carbonyl moieties. These polar groups actively interact with the functional sites of biopolymers through hydrogen bonding, electrostatic and hydrophobic interactions, and dipole–dipole forces.^{100,101} Such molecular interactions enhance cohesion and compatibility within the film-forming system, thereby improving its mechanical strength, structural integrity, and barrier properties and protecting food from microbial spoilage. In dry processing methods, the thermoplastic nature of biopolymers plays an important role in facilitating interactions with natural plant extracts. When the biopolymer is heated beyond its glass transition temperature during melt processing (such as extrusion, compression, or molding), it transitions into a flexible and elastic state. In this softened matrix, the mobility of the polymer chains increases, promoting the diffusion and uniform dispersion of natural extracts. This enhanced molecular mobility allows stronger intermolecular interactions, such

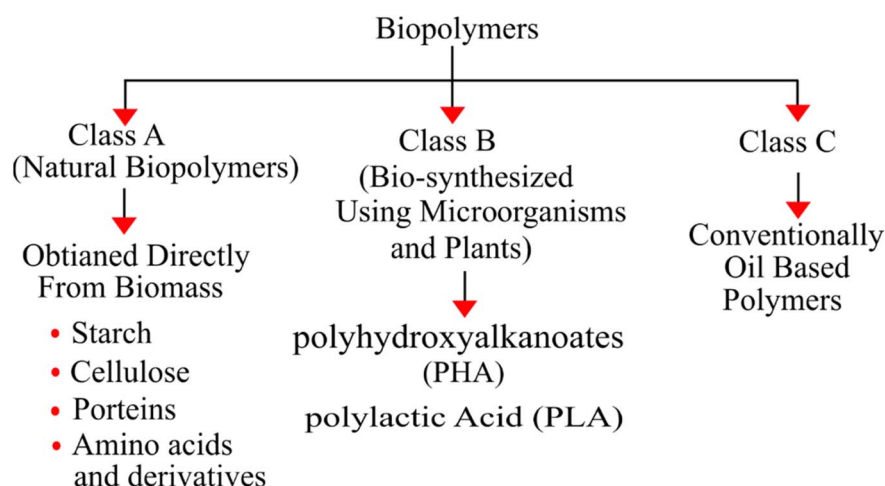


Fig. 2 Classification of biopolymers on the basis of their method of production.



as hydrogen bonding, hydrophobic associations, and van der Waals forces, between the biopolymer matrix and the active compounds of the extracts, leading to improved film integrity and functional performance during food processing.^{102,103} Vasconcelos *et al.*¹⁰⁴ fabricated cellulose acetate-based edible films incorporating flavonoid extracts. The results indicated that strong hydrogen bonding interactions between the functional groups of the biopolymer chains and those of the natural extracts led to increased intermolecular cohesion within the matrix. These interactions restrict the mobility of the polymer chains, resulting in a more compact and rigid network structure. Consequently, the tensile strength and elastic modulus were found to increase, whereas the elongation at break and water vapor permeability (WVP) decreased because of the reduced flexibility and tighter polymer-extract associations. On the other hand, Yong *et al.*¹⁰⁵ developed a chitosan-based edible film incorporating plant extracts (rambutan peel extract). The results of this study revealed that chitosan formed strong physical interactions with natural extracts, resulting in dense, more homogeneous and less crystalline microstructures. The improved interfacial compatibility between complex chitosan and the plant extract matrix enhanced chain mobility and network cohesion, resulting in films with increased elongation at break (EB) and tensile strength (TS). Moreover, light transmission, water vapor permeability, water solubility, and oxygen permeability were significantly reduced due to the formation of a denser and more compact polymer-extract matrix. Furthermore, the application of well-dispersed plant-based active compounds enhances antioxidant and antibacterial properties, as the stable network enables controlled release and retention of bioactivity. Zhang *et al.*¹⁰⁶ fabricated polysaccharide-based films incorporated with oregano essential oil. The outcomes of this study illustrated that hydrogen bonding and electrostatic interactions between the internal components of the film enhanced molecular cohesion and reduced surface polarity, thereby increasing the hydrophobicity of the film. Microstructural analysis of chitosan films containing *Sonneratia caseolaris* (L.) Engl. Compared with that of the control (without leaf extract), the surface of the leaf extract became rough and less compact. This may be because of the aggregation of extract compounds in the complex film matrix and the presence of numerous insoluble particles. These particles likely hinder uniform dispersion within the polymer matrix, indicating limited compatibility or weak interactions between the biopolymer chains and the bioactive compounds in the extract.^{107,108} Furthermore, a study conducted by Hasan *et al.*¹⁰⁹ investigated the interaction effects of the addition of extra virgin olive oil to sugar palm starch-chitosan films. The sugar palm starch-chitosan film exhibited the interaction mechanism of hydrogen bond formation. This formation occurs through the interaction effects between the NH^{3+} group present in the chitosan structure and the OH^- group present in starch molecules. Strong interactions were observed, which further reduced the number of free radical groups and the free volume in the complex polymer matrix. In addition, the oil in the sugar palm starch-chitosan film matrix exhibited hydrophobic and hydrophilic interactions, which thereby reduced the number of free

hydroxyl groups. The oil incorporated into films can also occupy the free volume between polymer chains, functioning as a plasticizing agent that enhances the flexibility and mobility of the polymer structure.¹¹⁰ Consequently, such molecular interactions influence several film characteristics, including thickness, thermal stability, mechanical performance, barrier efficiency, and morphological properties.

3.2.2 Strategies to enhance compatibility and performance. The enhancement of the compatibility and performance of biopolymer films incorporating natural extracts involves a combination of formulation, structural, and processing strategies. Polymer-extract compatibility can be improved through chemical modification or mild crosslinking to strengthen molecular interactions, whereas plasticizers and emulsifiers promote polymer chain flexibility and uniform dispersion of hydrophobic compounds.¹¹¹ Encapsulation techniques, such as nanoparticles, nanoemulsions, Pickering emulsions, liposomes, or cyclodextrin complexes, protect bioactive polyphenols from degradation, reduce volatility, and enable controlled or sustained release. The multilayer and composite film architectures further optimize retention and release by isolating active compounds in the inner layers while providing mechanical and barrier support externally. The incorporation of nanofillers such as cellulose nanocrystals or montmorillonite enhances tensile strength and barrier properties and regulates migration, whereas careful control of processing parameters, surface roughness, and hydrophilicity ensures homogeneous distribution and effective antimicrobial and antioxidant performance.¹¹² Collectively, these strategies allow biopolymer films to maintain desirable mechanical, barrier, and functional properties while improving the stability, retention, and efficacy of natural extracts for active food packaging applications.

The compatibility between bioactive compounds and biopolymer matrices is primarily determined by three major groups of factors: (i) the physicochemical properties of the components, (ii) the polar matrix characteristics, and (iii) the environmental and processing conditions.^{113,114} The physicochemical properties of the bioactive compounds and biopolymer matrices include polarity/hydrophilicity/hydrophobicity matching, hydrophobic-hydrophilic balance, solubility, volatility, affinity, chemical and structural interactions (stereochemistry, conformational flexibility, and molecular weight, size, and volume), and their ability to engage in specific intermolecular interactions. These interactions involve steric effects, electrostatic attractions or repulsion, and hydrogen bonding, all of which strongly influence the miscibility of lipophilic compounds with hydrophilic biopolymeric materials and their dispersion within the matrix. Polymer matrix characteristics such as crystallinity, free-volume distribution, glass transition temperature (T_g), and overall molecular mobility also strongly influence the degree of miscibility and prevent phase separation during film formation.¹¹⁵ The environmental and processing conditions, including pH, water activity, temperature, ionic strength, solvent type, component concentration and mixing ratio, also affect polymer-extract



interactions by altering molecular dynamics, solubility behavior, and dispersion efficiency.¹¹⁵

These compatibility factors are commonly evaluated using a range of advanced analytical techniques such as spectroscopic analyses (chemical interactions), thermal analyses (miscibility and molecular mobility), structural and morphological analyses (dispersion and microstructure), crystallinity and molecular organization, surface and interfacial analyses, and functional migration and release tests. Spectroscopic methods include Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), and mass spectrometry (MS).^{116–118} FTIR is employed to detect new hydrogen bonds or shifts in characteristic peaks that indicate chemical interactions. NMR reveals structural changes, molecular mobility, and chemical environment modifications, whereas MS confirms molecular identity and detects chemical changes after the incorporation of bioactive compounds. However, thermal analyses, including differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA), assess how the addition of bioactive compounds affects the thermal behavior of a polymer.¹¹⁹ DSC is used to monitor changes in the T_g or melting behavior, reflecting miscibility or plasticization effects, whereas TGA is used to monitor the thermal stability and verify whether thermal degradation occurs due to incompatibility. In addition, structural and morphological analyses (scanning electron microscopy (SEM), atomic force microscopy (AFM), and transmission electron microscopy (TEM)) are used to examine how uniformly the bioactive compound is distributed within the polymer matrix.^{119,120} SEM and AFM reveal surface and cross-sectional homogeneity and nanoscale surface topology and roughness, whereas TEM confirms particle encapsulation and dispersion at the nanoscale. Crystallinity and molecular organization determined *via* X-ray diffraction (XRD) analyses reveal whether bioactive incorporation disrupts or enhances polymer crystallinity.¹¹⁸ Additional tools, such as surface and interfacial analyses by contact angle measurements and surface energy analysis, are used to assess the interaction strength (hydrophilicity/hydrophobicity changes and interfacial compatibility) and wetting behavior (adhesion and affinity) between the polymer and the active compound, whereas migration assays and release kinetic studies provide functional evidence of how the compatibility of the polymer extract influences the stability, diffusivity, retention, and release of active compounds.

3.2.3 Potential trade-offs with film properties. Biopolymers such as polysaccharides, proteins, and lipids have attracted significant attention for their potential use in sustainable alternatives to petroleum-based plastics. Among them, polysaccharides, including cellulose, starch, chitosan, and carrageenan, are partially notable for their biodegradability and renewable nature. Similarly, lipid-based components such as wax and fatty acids, along with protein-based materials such as casein and soy protein, have exhibited superior performance in packaging applications. Despite these advantages, compared with conventional petroleum-based plastics, films derived from biopolymers generally possess lower mechanical strength and weaker barrier properties. To address these challenges,

researchers have focused on developing composite films by incorporating active natural plant-derived extracts to improve their overall functionality.¹²¹ In this context, Wang *et al.*¹²² introduced an innovative strategy to produce regenerated cellulose/curcumin composite films with Janus-type structures. Their method employed a pH-responsive dispersion process combined with one-sided hydrophobic modification. This approach was designed to address common drawbacks of conventional biopolymer packaging materials, including inadequate mechanical performance, high sensitivity to moisture, and suboptimal gas barrier properties. Most researchers have used an aqueous alkali/urea solvent approach for uniform and effective dispersion, which allows for homogeneous composite films with increased stability. A study by Periyasamy *et al.*¹²³ revealed that the incorporation of curcumin significantly improved the tensile strength. The maximum strength was 73 MPa, and the elongation at break was 28.5%, which is greater than that of many traditional biopolymer-based packaging materials. This improvement can be attributed to the reinforcing role of curcumin, which functions as a natural plasticizer, enhancing the flexibility of the film while preserving its tensile strength. However, the gas permeability (oxygen) of the film significantly decreased by 22.45%, which indicates a reduction in oxygen diffusion and increased food stability.

Another study by Akhter *et al.*¹²⁴ developed innovative functional packaging films by blending chitosan, pectin, and starch (0.75 : 1.5 : 0.75 w/w) and incorporating 0.5% rosemary essential oil, mint essential oil, nisin, and lactic acid. The results revealed that the incorporated rosemary and mint essential oils substantially improved the moisture barrier properties; mechanical properties, including tensile strength, Young's modulus, and percentage elongation at break; and thermal stability of the developed films compared with those of the control sample. The reduced tensile strength (7.72 MPa) observed in the control biocomposite film may be attributed to the presence of glycerol, whose hygroscopic nature introduces additional moisture into the polymer matrix, thereby weakening the tensile properties of the film.¹²⁵ The experimental results revealed that the tensile strength and elongation at break increased by 236.14% and 246.01%, respectively. The improvement in film strength following the incorporation of rosemary and mint essential oils can be attributed to their rich polyphenolic contents, such as carnosic acid, carnosol, rosmarinic acid, α -pinene, citronellol, and methyl eugenol, which are thereby capable of forming hydrogen bonds with the abundant polar hydroxyl groups in the chitosan–pectin matrix. Therefore, stronger bonding between the essential oils and the chitosan–pectin matrix may have tightened the polymer network, resulting in the films being more resistant to stress. This observation was consistent with previously reported studies in which clove oil was incorporated into citrus–pectin edible films.¹²⁶ Interactions between the carbohydrate polymer and essential oil created a cross-linked structure that restricted molecular mobility and improved tensile strength. Moreover, the mechanical strengths of the nisin extract- and lactic acid-containing protein-based and polysaccharide-based films did not differ significantly. Similar results were obtained for tea



extract-incorporated biocomposite films.¹²⁷ However, Akhter *et al.*¹²⁴ demonstrated that a biocomposite film without the addition of any extracts was clearer and more transparent. The incorporation of essential oils influenced the color parameters (L^* , a^* , b^*) and opacity of the biocomposite films. Specifically, the L^* and a^* values significantly decreased ($p \leq 0.05$) from 64.85 to 61.73 and -2.05 to -1.29 , respectively, whereas the b^* values did not significantly change, suggesting a shift toward a darker and slightly redder appearance. This alteration in optical properties may help protect packaged foods from visible and ultraviolet light, thereby reducing nutrient degradation, discoloration, and off-flavors.¹²⁸

3.3 Functional synergy

3.3.1 Controlled release and migration behavior. Active release packaging is an emerging food preservation approach in which functional agents, such as antioxidants or antimicrobials, are embedded within the packaging matrix rather than being applied directly to the food at high concentrations. In this system, the release of active compounds is carefully controlled through diffusion or degradation mechanisms, allowing the sustained migration of these agents from the packaging material to the food surface or its surrounding environment.¹²⁹ Unlike the material-related effects described above, this part specifically focuses on functional synergy, where the interaction between the biopolymer matrix and natural extracts directly governs release kinetics and antimicrobial efficacy.

Biodegradable polymers, such as starch, PLA, pectin, chitosan, gelatin, and cellulose derivatives, provide excellent carriers for such active agents.¹²⁹ Their physicochemical properties, such as hydrophilicity, crystallinity, and molecular integrity, significantly influence the migration rate and release kinetics of the active compounds. Compared with conventional nonbiodegradable plastic polymers, these biopolymers not only provide environmentally friendly alternatives but also enable adjustable release behavior through the modification of complex polymer compositions or incorporation of natural plant-derived extracts, essential oils, plasticizers, *etc.* Numerous studies have explored the use of these biopolymers in designing active packaging systems with controlled release profiles tailored to specific food preservation needs (Table 2). These controlled release phenomena represent the core functional advantages of biopolymer-extract systems, distinguishing them from simple material enhancements and making release kinetics the central mechanism that determines antimicrobial effectiveness.

The controlled release behavior of biopolymer extracts varies significantly across different food matrices.¹³⁰ This is due mainly to the physicochemical nature of the targeted food matrix, which influences both diffusion and the polymer network as well as solvent-polymer interactions and the molecular weight of the bioactive ingredient.¹³¹ High-fat foods generally promote faster release of hydrophobic/lipophilic bioactive compounds owing to enhanced partitioning into lipid phases, whereas low-fat or aqueous food matrices retain these molecules longer, resulting in slower diffusion. Moisture-

rich foods (water acts as a plasticizer) facilitate polymer swelling and increase the free volume within hydrophilic matrices, accelerating the mobility of active compounds, whereas dry or low-moisture foods restrict swelling and limit release.¹³⁰ In general, nonbiodegradable polymers exhibit diffusion as a dominant mechanism for molecular transport, whereas biodegradable polymers, swelling, and degradation or erosion are also involved.¹³² The pH and ionic strength of food also modulate release kinetics by altering the polymer charge, electrostatic interactions, and network structure.¹³³ For example, pH-sensitive polymers such as carboxymethyl chitosan, alginate, and chitosan exhibit markedly different release patterns: deprotonation under alkaline conditions increases electrostatic repulsion and swelling (thus faster release), whereas protonation at low pH can tighten the network and retard diffusion. Additionally, viscosity and matrix consistency influence molecular transport, with viscous or semisolid foods reducing migration compared with liquid foods. To describe and predict these release behaviors, several kinetic and mathematical models have been applied, including Fickian diffusion models based on concentration gradients, the Hixson-Crowell model for the dissolution rate, the Higuchi model for diffusion-controlled systems, the Baker-Lonsdale or modified Higuchi model for bioactive release from spherical matrices, the Korsmeyer-Peppas model for distinguishing between Fickian and non-Fickian mechanisms for bioactive release from polymeric matrices, Poiseuille's law of laminar flow for bioactive release from membrane matrices and the empirical Weibull and Peleg models to capture complex release patterns.^{130,141} Partition coefficient based models are also used to evaluate the distribution of active compounds between the film and food, especially in lipid-rich products. Together, these models help characterize release mechanisms and forecast performance across diverse food environments. For a better understanding of the mathematical models, detailed empirical and semi-empirical release kinetics models are illustrated in Table 3.

A study by Souza *et al.*¹³⁴ developed chitosan films incorporated with essential oil and hydroalcoholic extracts and reported that the migration of these films into fatty food simulant media occurred after 10 days of storage. The results of the migration assay indicated negligible diffusion toward a fatty food simulant, indicating strong retention within the polymer and limited release. These outcomes suggested that the active compounds within the extracts remained tightly bound to the complex polymeric matrix, indicating a greater affinity for the packaging material than for the fatty food simulant. In contrast, chitosan films with essential oils (EOs) exhibited an "exponential growth to a maximum" release profile, reaching equilibrium within 48 h of storage. The total phenolic content (TPC) released was proportional to the TPC in the crude EOs, with ginger and tea EOs resulting in greater release and rosemary, sage, and thyme EOs resulting in lower release. Most of the films, with the exception of the chitosan-ginger EO (0.92), had a release ratio of 0.34–0.52, reflecting differences in polymer-active compound interactions. These behaviors were satisfactorily associated with Fickian diffusion models, which present their interactions and concentration gradients in controlling release kinetics from





Table 2 Controlled release and migration behavior of biodegradable biopolymer-based active packaging

Source of biopolymer	Natural extract/active agent	Type of active compound	Release behavior/migration profile	Major findings	References
Chitosan	Essential oils (ginger, tea tree, rosemary, sage, thyme)	Phenolic compounds, terpenoids	Exponential growth to a maximum; equilibrium within 48 h	• Release proportional to phenolic content of EO; ginger EO showed highest diffusion (release ratio 0.92); behavior followed Fickian diffusion	Souza <i>et al.</i> ¹³⁴
	Hydroalcoholic extracts	Polyphenols	Negligible diffusion	• Strong interaction between polymer and extract; no detectable migration to fatty food simulant	
	Thyme essential oil	Thymol	Release rate varied with solvent polarity: slowest in 95% ethanol (up to 300 min), fastest in 50% ethanol	• The addition of polysaccharides (xanthan gum, pullulan, gum tragacanth, Arabic gum) altered essential oil release	
Chitosan-Arabic gum film (applied to nectarine)	Thyme essential oil (thymol)	Antifungal agent	Release of thymol vapor from film surface provided antifungal protection	• Arabic gum and pullulan delayed release in 95% ethanol due to stronger polymer-oil interactions	Lian <i>et al.</i> ¹³⁵
Chitosan (CS) and whey-based laminate	Cinnamon extract (encapsulated in CS nanoparticles)	Polyphenols/antioxidant and antimicrobial compounds	Similar desorption profile to ROS; ≈10–15% release after 24 h (within EU OML limit)	• Composite film effectively inhibited fungal growth on nectarine due to controlled release of thymol	
				• The coatings displayed high antioxidant activity (>90% in liquid; ~40% when coated); release dependent on chitosan-polyphenol interactions	
Polysaccharides film	EOs from various plant sources	Terpenes, terpenoids, phenols, aldehydes, alcohols, ketones, amines, and amides	Diffusion-controlled; dependent on EO miscibility with food matrix	• Better antifungal activity observed	Anis <i>et al.</i> ¹³⁷
Cellulose acetate	Pink pepper EO	Volatile terpenes and phenolic compounds	Migration observed at 4 °C over 12 days	• Extracts are key drivers of microbial inhibition	
				• EOs migrated from the matrix phase to the film surface and further contact with foods	
Cellulose nanofibers (CNF)	Purple sweet potato anthocyanins	Natural dye/pH indicator	Responds to pH changes from 2–12; color changes from red to yellow	• EOs levels significantly decreased within the films with the time	Dannenberg <i>et al.</i> , ¹³⁸ Tian <i>et al.</i> ¹³⁹
	Oregano essential oil (OEO)	Antimicrobial agent	Incorporated into film; likely gradual antimicrobial effect	• The lipid components of cheese facilitated EO migration from the polymer matrix into the food	
	Anthocyanins + oregano essential oil	pH indicator + antimicrobial	Not explicitly stated; pH-sensitive and antimicrobial properties combined	• pH-sensitive release observed <i>via</i> color change	
				• Antimicrobial effect likely due to gradual release of OEO	Chen <i>et al.</i> ¹⁴⁰
				• Combined functionality observed; pH-sensitive color change and antimicrobial activity indicate active agents are effectively incorporated, but specific migration behavior not detailed	

active packaging. However, the migration behavior of active compounds is influenced by various factors, including (a) polymer characteristics such as morphology, crystallinity, molecular weight, and microcavity distribution, which affect diffusion pathways; (b) the physicochemical characteristics of active agents, including polarity, solubility, and molecular structure; and (c) the interactions between the polymer and the incorporated compounds, such as plasticizing or anti-plasticizing effects.¹⁴² Environmental factors also exert strong control over release rates, with moisture being particularly influential. Water acts as a plasticizer in hydrophilic biopolymers, disrupting intermolecular hydrogen bonding and increasing the free volume within the matrix. This expansion of chain spacing within the polymer matrix accelerates the diffusion of entrapped active compounds, increases mobility and can dramatically shift films from glassy to rubbery states. For example, the diffusion coefficient of carvacrol (a natural monoterpenoid phenol) in soy protein films increased from $0.11 \times 10^{-16} \text{ m}^2 \text{ s}^{-1}$ at 60% RH to $7.5 \times 10^{-16} \text{ m}^2 \text{ s}^{-1}$ at 100% RH.¹⁴³

However, pH-responsive polymers, such as carboxymethyl chitosan and alginate, also exhibit adjustable release behavior. Under alkaline conditions, deprotonation of carboxyl groups increases electrostatic repulsion and matrix swelling, enlarging the pore size and promoting faster release of active compounds. A similar pattern of release kinetics was observed in hydrophilic pectin-based films¹⁵³ and chitosan-based films.¹⁵⁴ However, the concentration of EOs released in a controlled manner from food is important and governs the shelf stability of foods. At any point during storage, the concentration of EOs at the food surface should remain above the minimum inhibitory concentration (MIC) required to suppress microbial growth. A previous study by Anis *et al.*¹³⁷ suggested that the rapid diffusion rate may cause the EO concentration to drop below the MIC too quickly, resulting in detrimental effects on the antimicrobial properties and shelf-life of the EO. Additionally, if the diffusion rate is too slow, the EO concentration at the food surface may not reach an effective antimicrobial level. In this case, biopolymers incorporated with EO films may not be capable of protecting food. An earlier study by Souza *et al.*¹⁵⁴ demonstrated that the quantities of glycerol and EOs were constant and that variations in their migration profiles are attributed mainly to differences in the chemical composition and polarity of the EOs. The greater release observed for ginger EO may result from its greater solubility in ethanol (95%) and the lipophilic nature of its active constituents, as also noted by Reis *et al.*¹⁵⁵

Importantly, controlled release systems are closely coupled with migration safety considerations, as bioactive compounds can be transferred from packaging materials into food systems. Previously published literature emphasized that migration must remain within regulatory limits and must not generate toxicologically relevant concentrations.¹⁵⁶ Safety evaluation basically involves quantifying the amount of migrated compound *via* validated analytical methods such as chromatographic methods (HPLC or GC-MS), FTIR spectroscopy, NMR spectroscopy, thermal analyses such as DSC and TGA, electrochemical methods, and release cell systems (*e.g.*, Franz

diffusion cells), followed by comparisons with specific migration limits (SMLs) set by regulatory agencies.¹⁵⁶ On the other hand, the biological activity of the released compounds is typically evaluated through antimicrobial assays, including MIC determination and agar diffusion tests, ensuring that the released concentration remains microbiologically effective throughout storage.¹⁵⁷ These assessments also examine whether the release results in the formation of any degradation or reaction byproducts and confirm that all the incorporated natural extracts or essential oils are food-grade or GRAS substances. Migration testing is often carried out using standardized food simulants under controlled conditions of time, temperature, humidity, and pH to mimic realistic storage environments, ensuring that the packaging maintains both functional performance and consumer safety.¹⁵⁸

4. Advances in the fabrication of biopolymer-based antimicrobial films

The fabrication method plays a key role in determining the scalability, processing stability, and release behavior of biopolymer-based antimicrobial films. Rather than focusing only on formulations or lab steps, an engineering-oriented approach considers how factors such as temperature, shear, drying rate, solvent removal, and layer formation influence film uniformity, mechanical strength, diffusion pathways, and industrial applicability. The following subsections examine major fabrication techniques from this engineering perspective, highlighting how each method affects production efficiency, large-scale manufacturing potential, and the controlled release of antimicrobial agents.

4.1 Film-forming techniques

Different film-forming methods influence the processing stability, scalability, and release behavior of antimicrobial agents in biopolymer films. Each technique produces a distinct microstructure, which affects the mechanical strength, barrier properties, and diffusion pathways. Solvent casting is widely used in laboratory studies because it allows easy mixing of polymers, plasticizers, and active compounds. However, it is difficult to scale due to long drying times, high energy use, and sensitivity to temperature and humidity. Uneven solvent evaporation can create variable film thicknesses and internal micro voids, which lead to inconsistent mechanical properties and unpredictable release rates of antimicrobial agents.¹⁵⁹ For example, chitosan films prepared by solvent casting with a thyme-oil nanoemulsion (TH-NE) exhibited enhanced antimicrobial activity and increased flexibility, attributed to their greater elongation at break. However, achieving thicker, uniform films requires prolonged drying at elevated temperatures (50–55 °C for 24 hours), conditions that limit industrial feasibility.¹⁶⁰ In another study on a solvent-cast system, chickpea-whey protein films enriched with quercetin demonstrated how formulation changes can influence film structure and release behavior. Increasing the whey protein content strengthened intermolecular interactions and improved the





Table 3 Empirical and semiempirical release kinetics models, parameters, and their applications

Model	Equation	Model parameters	Application	References
Zero order	$M_t = M_0 - k_0t$	M_0 : initial concentration of encapsulated substance	<ul style="list-style-type: none">• Describing release kinetics of matrix systems slabs, low solubility, or coated material, osmotic systems• Not common mechanism due to rapid dissolution of most food materials	Malekjani & Jafari ¹³²
First order	$M_t = M_0e^{-k_1t}$	M_t : concentration at any time t k_0 : zero-order equation rate constant (concentration per time) k_1 : first-order equation rate constant (time ⁻¹)	<ul style="list-style-type: none">• Best describes the release kinetics of water-soluble material in porous matrices and ionizable oil or water-soluble materials from W/O/W emulsions	Malekjani & Jafari ¹³²
Higuchi model	$f_1 = M_t = K_Ht^{0.5}$	K_H : dissolution constant (concentration/time ^{0.5})	<ul style="list-style-type: none">• Best suited for modeling the release of water-soluble or low solubility encapsulated compounds from solid or semisolid matrices	Higuchi ¹⁴⁴
Hixson-Crowell model	$M_0^{1/3} - M_t^{1/3} = K_{HC}t$	K_{HC} : model constant incorporating the surface volume relation (concentration/time ^{1/3})	<ul style="list-style-type: none">• Applicable for planar systems where dissolution occurs uniformly across surfaces parallel to the active compound	Hixson & Crowell ¹⁴⁵
Ritger-Peppas and Korsmeyer-Peppas model (power law)	$\frac{M_t}{M_\infty} = kt^n$	$\frac{M_t}{M_\infty}$: released fraction at any time t k : rate constant influenced by system structure and geometry (release velocity constant) K : model constant	<ul style="list-style-type: none">• Applied when release mechanism is not known or when more than one release mechanism is involved	Korsmeyer <i>et al.</i> , ¹⁴⁶ Ritger & Peppas ¹⁴⁷
Baker-Lonsdale or modified Higuchi model	$f_1 = \frac{3}{2} \left[1 - \left(1 - \left(\frac{M_t}{M_\infty} \right)^{2/3} \right) \right] - \frac{M_t}{M_\infty} = Kt$		<ul style="list-style-type: none">• Suitable for microspheres or microcapsules• Bioactive release from spherical matrices	Baker & Lonsdale ¹⁴⁸
Weibull model	$m = 1 - \exp \left[\frac{t - T_i}{a} \right]$	a : scale parameter (determines the process time scale) T_i : location parameter indicating lag time before release begins (often zero)	<ul style="list-style-type: none">• Suitable for wide range of release situations and is useful for comparing release profiles in matrix systems	Weibull ¹⁴⁹
Hopfenberg model	$\frac{M_t}{M_\infty} = 1 - \left[1 - \frac{k_0t^n}{c_0a_0} \right]^n$	c_0 : initial concentration a_0 : radius (sphere or cylinder radius, or half-thickness for slab) k_0 : erosion rate constant n : for slab, cylinder, and the sphere is 1, 2, and 3, respectively	<ul style="list-style-type: none">• Applicable for modeling optimized oil spheres that show site specific, biphasic release behavior	Katzhendler <i>et al.</i> ¹⁵¹



Table 3 (Contd.)

Model	Equation	Model parameters	Application	References
Poiseuille's law of laminar flow	$\frac{dM}{dt} = \frac{\pi r^4}{8\eta h} (P_1 - P_2)$	$\frac{dM}{dt}$: drug/bioactive release rate c : solute concentration inside the matrix r : radius of the cylindrical pore/orifice η : viscosity of the fluid (matrix or carrier) h : membrane thickness (length of channel) $P_1 - P_2$ = pressure difference across the membrane	—	Bayer ¹⁴¹
Peppas-Sahlin model	$\frac{M_t}{M_\infty} = K_1 t^n + K_2 t^{2m}$	K_1 and K_2 , are constants m : exponent representing the Fickian diffusion contribution based on the system's geometry $K_1 t^n$ term represents the Fickian diffusion contribution and $K_2 t^{2m}$ represents the polymer relaxation contribution	<ul style="list-style-type: none">• Useful to calculate the approximate two contribution mechanisms such as diffusional and relaxational in an anomalous release process	Peppas & Sahlin ¹⁵⁰
Brazel and Peppas model	$\text{De} = \frac{\lambda}{\theta} = \frac{\lambda D_{1,2}}{(\delta(t))} = \frac{\text{relaxation time}}{\text{time of solvent diffusion}}$	λ : relaxation time of the polymer θ : characteristic water-diffusion time in a swellable matrix $\frac{\delta^2}{D_{1,2}}$: ratio of diffusing distance to diffusing coefficient of water in the polymer	<ul style="list-style-type: none">• Useful for analyzing how water uptake and polymer swelling influence drug release in swellable or erodible matrices• Helps compare the relative rates of water diffusion and polymer relaxation to optimize controlled-release formulations	Bruschl ¹⁵²

barrier and mechanical performance up to 40%, whereas increasing the whey protein content caused structural defects. The films also showed medium-dependent quercetin release, highlighting how casting-based formulations allow tuning of the microstructure and controlled release through adjustment of the protein composition.¹⁶¹

Extrusion provides a more stable and continuous process and is currently the most industry-ready technique. Controlled heat and mechanical shear during extrusion allow precise structuring of the polymer matrix, resulting in uniform films with consistent performance.¹⁶² Co-extrusion enables multilayer films where barrier layers can be engineered to regulate moisture transport and slow the release of active agents. Studies on alginate-based films have shown that plasticizer content strongly affects melt viscosity and thermal stability and that higher glycerol levels help maintain mechanical stability even at elevated processing temperatures.¹⁶³ Nanocomposite films produced by melt-mixing extrusion also demonstrate how extrusion can improve processing stability and film performance. For example, mLLDPE films reinforced with graphene nanoplatelets (GNPs) showed excellent filler dispersion and more stable processing, with certain GNP levels reducing torque and pressure during extrusion. The GNPs acted as nucleating agents, increasing the crystallinity and enhancing the oxygen and water-vapor barrier properties while also increasing the Young's modulus. These results show that extrusion can effectively incorporate nanofillers and tailor the film structure and barrier behavior in a scalable manner.¹⁶⁴ Twin-screw (multi-screw) extrusion is often preferred over single-screw systems because the intense shear in single-screw extrusion can generate excess heat, which may degrade thermally sensitive antimicrobial compounds. In contrast, the more controlled, positive-displacement flow in multiscrew extrusion helps reduce thermal hotspots and better preserves the activity of thermolabile bioactive agents.¹⁶⁵ In addition to food packaging, advances in additive manufacturing, particularly material extrusion, have shown that similar extrusion-based processes can be adapted for high-precision applications such as printable electronics and bioengineered scaffolds. These systems rely on the formulation of printable inks from colloidal suspensions, polymer melts, and biopolymer-based composites with tunable mechanical and functional properties. Insights from this field highlight the broader versatility of extrusion, including its compatibility with a wide range of composite formulations and active materials (e.g., nanomaterials, sensors, or bioactives), which may inform the next generation of functional and responsive packaging films.¹⁶⁶ Extrusion not only supports higher throughput but also avoids solvent-related variability and maintains structural integrity during long-term storage, whereas solvent-cast films are more sensitive to humidity-induced recrystallization and mechanical weakening.¹⁶⁷

Electrospinning is a versatile technique used to fabricate nanofiber-based films for active food packaging applications. A typical electrospinning setup consists of four main components: a high-voltage power supply, an injection pump, a capillary tube (or spinneret), and a grounded metal collector. In this

process, a polymer solution is loaded into the capillary tube, and a high-voltage electric field is applied between the needle tip and the collector. The electric field induces the formation of a Taylor cone, from which a charged jet of the polymer solution is ejected. As the jet travels toward the collector, the solvent rapidly evaporates, resulting in the deposition of solid nanofibers on the surface.¹⁶⁸ Electrospinning produces nanofibrous films with very high surface areas and porosities, which can enhance antimicrobial effectiveness because the active agents can diffuse more easily. It is also useful for encapsulating sensitive bioactive compounds within nanofibers.¹⁶⁹ For example, electrospun nanofiber mats made from cold-water fish gelatin supplemented with up to 20% bovine lactoferrin showed significant antimicrobial activity against several foodborne pathogens, including *Pseudomonas fluorescens* and *E. coli*.¹⁷⁰ Although high bioactive loading reduced the fiber diameter and tensile strength, the system demonstrated strong potential for active packaging applications. Despite its advantages, electrospinning faces several technical limitations. The method is highly sensitive to the solution viscosity, voltage, humidity, and temperature. These variations make continuous, large-scale production difficult. Traditional single-needle electrospinning has low throughput, and although newer multi-nozzle or free-surface systems improve productivity, industrial adoption is still limited. Thus, electrospinning offers excellent functional performance but currently lacks the scalability required for mass production.¹⁷¹

4.2 Layer-by-layer and coating technologies

Compared with single-layer films, multilayer and coating-based fabrication techniques provide much better control over the film structure and release behavior. From a process-engineering perspective, these methods allow the precise adjustment of layer thickness, interfacial interactions, and drying dynamics, which directly influence processing stability, scalability, and antimicrobial release profiles. Techniques such as layer-by-layer (LbL) assembly, dip coating, spray coating, and roll coating enable the formation of films with tailored barrier properties and controlled diffusion pathways, supporting the development of high-performance and scalable biopolymer films.¹⁶⁸

4.2.1 Layer-by-layer assembly. The layer-by-layer (LbL) technique is an advanced assembly method used for fabricating biodegradable and edible films through the alternate deposition of oppositely charged biopolymers, such as polysaccharides, proteins, and lipids. This approach allows precise control over film thickness, permeability, and functional properties, enabling the creation of customized coatings for food preservation. In food packaging, LbL films provide excellent gas and moisture barrier properties, enhanced adhesion, and tunable release of active compounds. For example, Poverenov *et al.*¹⁷² developed a bilayer coating of alginate (inner negatively charged layer) and chitosan (outer positively charged antimicrobial layer) for fresh-cut melons, which effectively reduced microbial counts by two orders of magnitude and minimized moisture loss, demonstrating the synergistic benefits of the multilayer design. Similarly, Brasil *et al.*¹⁷³ employed multilayer



chitosan coatings incorporating *trans*-cinnamaldehyde/ β -cyclodextrin complexes on fresh-cut papaya, resulting in an extended shelf-life of up to 15 days and substantial inhibition of microbial growth. These examples highlight how LbL technology enables the incorporation of natural antimicrobial agents within biodegradable films, providing sustainable alternatives to synthetic packaging while improving food safety and storability. Overall, the LbL approach represents a promising strategy for the development of active biodegradable packaging materials with enhanced structural integrity and antimicrobial efficacy.^{172,174}

4.2.2 Coating techniques. Among edible coating deposition methods, dipping and spraying are the most widely adopted owing to their operational simplicity and adaptability for diverse food substrates. The dipping method involves immersing the food product into a film-forming dispersion, followed by drainage and solvent evaporation, producing a uniform coating layer. Dip coating consists of three sequential stages—immersion and dwelling, deposition, and evaporation—where the viscosity, surface tension, and withdrawal rate of the solution affect the final coating thickness.¹⁷⁵ As reviewed by Suhag *et al.*,¹⁷⁶ dipping has been effectively applied to various products, including mangoes, tomatoes, guavas, papayas, and fish fillets, to reduce microbial spoilage and moisture loss while maintaining color and firmness. However, this method can produce thicker coatings that restrict gaseous exchange, occasionally inducing anaerobic respiration and off-flavor development during storage.¹⁷⁷ Additional limitations include dilution of coating solutions, microbial contamination in dipping vats, and removal of natural fruit waxes, which may reduce surface gloss. Despite these drawbacks, dipping remains a preferred laboratory-scale technique because it ensures complete coverage of irregular surfaces and requires minimal specialized equipment.^{176,178} The spraying technique is one of the most versatile and widely employed methods for applying edible and biodegradable coatings onto food surfaces. In this process, the coating solution is atomized into fine droplets, typically within the micrometer to nanometer range, using an atomizer and then evenly deposited over the substrate. The efficiency and quality of the resulting film depend on several factors, including the rheological properties of the coating dispersion (such as viscosity, surface tension, and temperature) and the drying parameters (method, time, and temperature), which collectively influence coating uniformity, adhesion, and final surface characteristics.¹⁷⁹ An active pectin-CMC composite film containing oleic acid, calcium propionate, and silver-loaded zeolite microparticles was recently developed as a coating for bread packaging. Rheological analysis revealed that the film-forming solution had a suitable viscosity and flow behavior for scalable coating processes such as spraying or paper coating. The resulting films exhibited strong anti-mold activity and improved oxygen and CO₂ barrier properties while releasing only small, regulated amounts of Ag⁺ and CaP, demonstrating controlled release behavior.¹⁸⁰ This shows how formulation design and coating-process compatibility can be combined to create functional, scalable antimicrobial packaging systems without relying on nanoparticles.

In industrial and research applications, spraying can be achieved through air spray atomization, air-assisted airless atomization, or pressure atomization, each of which differ in how compressed air and pressure generate droplet formation. The nozzle configuration—pneumatic or hydraulic, with designs such as a solid stream, hollow cone, flat spray, or full cone—plays a critical role in determining droplet size, deposition pattern, and coverage.¹⁸¹ This method facilitates the formation of thin, uniform, and continuous coatings without excessive material waste, offering a distinct advantage over immersion-based techniques.

Several studies have demonstrated the potential of spraying in enhancing food preservation. For example, Ribeiro *et al.*¹⁸² reported that carrageenan-based coatings containing calcium chloride, when sprayed on strawberries, effectively reduced microbial growth and maintained fruit firmness during refrigerated storage. Similarly, Saberi *et al.*¹⁸³ reported that a pea starch-guar gum-lipid composite coating containing shellac and oleic acid delayed firmness loss and minimized ethylene production in oranges, highlighting the ability of spray-coated films to increase postharvest quality. Furthermore, spray coating has been successfully employed for chitosan-, gelatin-, and starch-based coatings on fruits, vegetables, and meat products, where it enhances their mechanical stability, barrier properties, and shelf life.¹⁷⁶

From a process engineering standpoint, the atomization pressure and droplet size are critical parameters influencing coating homogeneity and film integrity. Excessive pressure (above approximately 3.5 bar) can disrupt the film-forming network, resulting in nonuniform surface coverage or droplet rebound. The technique also supports multilayer and composite applications, such as alternating sprays of alginate and calcium chloride to produce cross-linked gel layers, which provide improved water resistance and mechanical strength.¹⁷⁹ Compared with dipping, the spraying method offers superior controllability, reduced drying time, and lower solution waste, making it highly suitable for continuous and automated coating lines. Overall, spray coating represents an efficient, scalable, and adaptable approach for fabricating biopolymer-based edible films with enhanced barrier, antimicrobial, and mechanical properties that meet the requirements of modern sustainable packaging systems.

4.3 Nanotechnology-enabled approaches

The integration of nanotechnology into biopolymer-based packaging has revolutionized the development of antimicrobial films by enhancing their physicochemical, mechanical, and functional properties. Nanomaterials, typically in the 1–100 nm range, impart unique characteristics such as a high surface area, tunable reactivity, and enhanced barrier strength that are not achievable with conventional fillers. The incorporation of nanosized reinforcements, including metallic nanoparticles (AgNPs, ZnO NPs, TiO₂ NPs), nanoclays, and biogenic nanocellulose, into biodegradable polymer matrices such as chitosan, starch, and polyvinyl alcohol significantly improves the tensile strength, gas impermeability, and antimicrobial



efficiency. These improvements arise from the creation of tortuous diffusion paths and dense interfacial bonding networks within the polymer matrix, which effectively restrict oxygen and moisture permeation while allowing the controlled release of antimicrobial agents.¹⁸⁴ Among metallic nanofillers, silver nanoparticles are the most extensively used owing to their strong antibacterial activity against both Gram-positive and Gram-negative bacteria, which is achieved through membrane disruption and reactive oxygen species (ROS) generation.¹⁸⁵ Moreover, compared with metallic silver, zinc oxide and titanium dioxide nanoparticles exhibit photocatalytic antimicrobial action, offering lower toxicity and improved stability.¹⁸⁴

Recent studies have highlighted the use of hybrid nanostructures that combine nanoparticles with natural bioactive compounds to achieve synergistic antimicrobial and antioxidant effects. For example, cellulose-based nanocomposite films incorporating curcumin and modified with organosilane exhibited a Janus architecture with asymmetric hydrophilic–hydrophobic surfaces, enhancing UV resistance, moisture barrier, and antioxidant activity while enabling visual freshness monitoring and maintaining full biodegradability.¹⁸⁶ A study on PVA–chitin nanofibril nanocomposites revealed that incorporating 10% fungal-derived nanofibrils reduced water vapor permeability by nearly 70% and significantly increased film stiffness, demonstrating the strong reinforcing potential of biobased nanofillers. Because fungal chitin provides a renewable feedstock, this approach also supports environmentally sustainable packaging, as confirmed by life cycle assessment. These results highlight how nanoscale fillers can simultaneously enhance the mechanical, barrier, and sustainability performance of biopolymer films. Another study demonstrated that a multilayer film incorporating electrospun zein nanofibers loaded with cumin essential oil (CEO) revealed how nanoscale structures can enhance film performance and control release behavior. Increasing the nanofiber layer thickness improved the mechanical strength, barrier properties, crystallinity, and thermal stability, while the FESEM images confirmed a more compact structure. The films also exhibited stronger antimicrobial activity and sustained CEO release in aqueous medium, demonstrating the effectiveness of nanofiber-based architectures in tuning both functionality and controlled diffusion.¹⁸⁷

In addition to inorganic nanostructures, organic nanomaterials such as cellulose nanocrystals (CNCs) and chitosan nanoparticles (CSNPs) have gained substantial attention because of their compatibility and biodegradability. Their high crystallinity, abundant hydroxyl groups, and ability to form strong hydrogen bonds facilitate uniform dispersion within polymer matrices, resulting in enhanced mechanical integrity and moisture barrier properties. For example, CNC-reinforced chitosan films demonstrated an improved tensile modulus and reduced water vapor permeability due to the formation of dense interfacial hydrogen-bonding networks between cellulose nanocrystals and the chitosan matrix.¹⁸⁸ In a previous study, chitosan–ZnO nanocomposite coatings on LDPE films effectively reduced bacterial (by 63%) and fungal (twofold) growth on okra during 12 days of storage at 25 °C without affecting quality parameters. This demonstrates their strong antimicrobial

efficiency and suitability for active food packaging applications.¹⁸⁹ Moreover, nanocomposite films fabricated *via* solution casting or electrospinning have shown superior antimicrobial performance when integrated with natural bioactive agents such as essential oils, polyphenols, or curcumin nanoparticles. These bioactives can be encapsulated within nanoemulsions or nanoliposomes to prevent volatility and degradation, allowing for controlled release at the food–film interface and extended antimicrobial efficacy.¹⁸⁴

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Despite these advantages, safety and regulatory concerns regarding nanoparticle migration into food remain significant. The potential leaching of metallic nanoparticles such as AgNPs and ZnONPs into food matrices may pose toxicity risks, necessitating detailed migration studies and risk assessments.¹⁸⁶ Consequently, biogenic or green synthesis approaches using plant extracts or microbial routes are increasingly favored to minimize toxicity and ensure compliance with safety standards.¹⁹⁰ The future of nanotechnology-enabled food packaging lies in the design of multifunctional nanocomposite films that combine mechanical strength, biodegradability, and intelligent sensing capabilities, enabling real-time freshness monitoring while ensuring environmental sustainability. These innovations are paving the path toward the next generation of smart, bioreponsive, and fully compostable food packaging systems, which are aligned with circular economy principles and global sustainability goals.

5. The “intelligent” and “integrated” antimicrobial package

Smart packaging can be broadly classified into two main types: active packaging and intelligent packaging. By releasing antimicrobial agents, moisture regulators, and gas scavengers, active packaging can interact dynamically with a food product or its surrounding environment to improve preservation.¹⁹¹ Conversely, intelligent packaging incorporates sensors and indicators such as biosensors, time–temperature indicators (TTIs), and gas detectors to continuously monitor the freshness and quality of food in real time.¹⁹² Recent developments increasingly combine these two functionalities, creating integrated, multifunctional systems in which a single packaging matrix is capable of both preserving food and reporting its status, often through pH-, enzyme-, or volatile-responsive mechanisms. These systems provide critical data on spoilage, contamination, and storage conditions, enabling timely interventions to increase food safety and reduce waste.¹⁹³ Additionally, known as smart packaging, intelligent packaging



specifically detects certain attributes of a food or its environment and conveys this information to manufacturers, distributors, and consumers.¹⁹⁴ These pH- and volatile-responsive indicator systems have recently been enhanced by combining indicators with antimicrobial agents within the same matrix. Recent examples include chitosan/gelatin/ZnO composite films loaded with pomegranate flower anthocyanin nanocapsules for fish freshness monitoring¹⁹⁵ and active chitosan films containing single and double nanoemulsions of *Oliveria decumbens* essential oil with eggplant anthocyanins for chicken preservation,¹⁹⁶ highlighting the emerging trend of combining antimicrobial efficacy with real-time, colorimetric freshness indications.

5.1 Microbial contamination and active packaging

Microbial contamination, which potentially occurs at any stage of the supply chain, remains a significant challenge for food safety and shelf-life. Such contamination can degrade sensory qualities or pose serious health hazards to consumers.¹⁹⁷ Antimicrobial active packaging involves the embedding of controlled-release compounds that inhibit microbial growth, regulate humidity, and prevent spoilage.¹⁹⁸ Biodegradable films serve as sustainable carriers for antimicrobial agents and are often enhanced with bioactive compounds such as essential oils and phenolics to increase their antimicrobial and antioxidant capacity. Biopolymer-based biodegradable packaging, including protein-derived films, is increasingly favored as an eco-friendly alternative to conventional plastics. The incorporation of antimicrobial substances such as essential oils or chitosan composites into these materials has proven effective in prolonging product freshness and shelf life.¹⁹⁴ Chitosan, which has inherent antimicrobial properties, inhibits microbial proliferation and helps stabilize the microenvironment within packaging. Combining chitosan with essential oils, metallic nanoparticles, or natural phenolic compounds results in synergistically improved pathogen resistance and food preservation.¹⁹⁴ For example, multifunctional biocomposites fabricated by integrating biosynthesized silver nanoparticles (AgNPs) and grapefruit peel extract into polyvinyl alcohol (PVA) matrices exhibit robust antimicrobial effects against foodborne pathogens, coupled with antioxidant activity, suggesting a promising, cost-effective, and environmentally sustainable packaging platform.¹⁹⁹

5.2 Metal-based antimicrobial packaging

Metals such as silver (Ag), copper (Cu), and zinc (Zn) are the most frequently used elements for antimicrobial meat packaging because their effective microbial inhibition is balanced with considerations of cost and safety. Antimicrobial action primarily involves the release of metal ions. For example, Ag⁺ ions interact with bacterial proteins by forming bonds with thiol groups, disrupting electron transport, and inhibiting DNA replication.²⁰⁰ However, single-metal systems pose limitations; silver ions are susceptible to oxidation, diminishing their efficacy, and copper ions may alter the visual appearance of packaging materials. To overcome these challenges, recent research

has focused on combining or doping multiple metals to enhance antimicrobial activity through synergistic effects and improved physicochemical properties. Nonetheless, issues surrounding metal ion migration and potential toxicity require rigorous assessment to guarantee safe application in direct food contact.²⁰¹ Currently, comprehensive toxicity evaluation frameworks for metal antimicrobial agents in meat packaging remain underdeveloped.

5.3 Bacteriocins and regenerated cellulose-based packaging

Historically, regenerated cellulose-based materials, such as cellophane, have been valued for their mechanical integrity, transparency, and antimicrobial capabilities, especially when combined with agents such as nisin.²⁰² Bacteriocins, bacterially produced ribosomal peptides, are increasingly recognized as natural antimicrobial agents because of their resistance to heat and acidic environments.⁷³ Nisin, for example, acts through electrostatic interactions with the negatively charged bacterial membrane, leading to cellular leakage and death. It is notably effective against Gram-positive bacteria such as *Micrococcus luteus*, *Staphylococcus aureus*, and *Bacillus cereus* and is often incorporated into polymer-based packaging to increase meat safety.^{203,204}

5.4 Biopolymer-based antimicrobial packaging films

Biopolymer-based antimicrobial packaging films (BPPFs) have demonstrated efficacy in inhibiting spoilage organisms through controlled release strategies, thus improving shelf-life and safety. However, industrial adoption faces challenges, including higher production costs than synthetic plastics, the migration of components exceeding permissible limits, diminished effectiveness under refrigeration, and potential microbial resistance arising from biofilms or horizontal gene transfer.²⁰⁵ Emerging antimicrobial technologies such as photodynamic inactivation (PDI) harness a photosensitizer activated by light and oxygen to generate reactive oxygen species (ROS), which selectively kill microbes. PDI offers broad-spectrum antimicrobial action, rapid targeting of microbial cells, efficient inactivation under mild conditions, and a low propensity for inducing resistance, regardless of the microbial antibiotic profile.^{205,206} For example, curcumin (CUR), a widely investigated photosensitizer in PDI, also serves as an antioxidant and cross-linking agent in biopolymeric films. CUR-incorporated films exhibit strong antibacterial effects against various pathogens and extend the shelf-life of foods such as grapes and pork by generating ROS that suppress microbial growth.^{205,207} Composites containing both CUR and ϵ -polylysine, which are natural antimicrobial polymers recognized as safe by the FDA, show improved water resistance and prevent the spoilage of refrigerated seafood by bacteria.²⁰⁸ Encapsulation of CUR within polymer matrices enhances its photostability and antibacterial efficacy under illumination.²⁰⁹ Riboflavin (RB) has emerged as a cost-effective alternative to CUR, exhibiting superior acid and thermal stability. RB-incorporated chitosan films show improved mechanical strength, antimicrobial activity, and





Table 4 Application and effect of biopolymer- and natural extract-based antimicrobial packaging

Antimicrobial extract	Biopolymers	Application	Major findings	References
Zein-magnolol	Sodium carboxymethyl cellulose	Fresh-cut jackfruit	Minimizes weight loss, retains flavor, and delays the ripening process for 8 days at 25 °C	Wei <i>et al.</i> ²²⁶
Genipin	Chitosan, and gelatin	Strawberries	Better firmness and soluble solids content up to 5 days	Li <i>et al.</i> ²²⁷
Chlorogenic acid	Agar	Cherry	Spoilage rate reduces to 12.5% after 9 days at 25 °C with 75% relative humidity	Han <i>et al.</i> ²²⁸
Phenolic-rich substance from white and red varieties Lemongrass oil	Poly lactic acid	Sunflower oil	After 30 days, peroxide index was lesser	de Freitas <i>et al.</i> ²²⁹
Apple polyphenols	Polyvinyl alcohol	Gimbap	Microbial growth was very less after 12 h at 25 °C with 90% of relative humidity	Ahn <i>et al.</i> ²³⁰
Diacetyl	Gelatin, carboxymethyl cellulose, and chitosan	Beef	Prevent inhibition of <i>E. coli</i> and <i>S. aureus</i> , up to 3 days at 4 °C	Ahmad <i>et al.</i> ²³³
Berberine	Sodium stearate and cyclodextrin nanosponges	Beef	More than 90% of inhibition rate against <i>Pseudomonas</i> spp. and lactic acid bacteria after 11 days at 4 °C	Rupérez <i>et al.</i> ²³⁴
Microalgal	Gelatin, dialdehyde nanocellulose, and ε-polylysine	Pork	Delaying spoilage by reducing TVBN and TBARS for 10 days at 4 °C	Xu <i>et al.</i> ²³⁵
Cinnamaldehyde	Pectin and gelatin	Chicken meat	<i>E. coli</i> and <i>S. aureus</i> inhibition rate were 80 and 20%, respectively, after 7 days	Karabulut & Goksen ²³⁶
Betalain	Chitosan, PVA and gelatin	Trout fillets	Under 4 °C, no qualitative changes till 12 days	Hosseini <i>et al.</i> ²³⁷
Curcumin	Starch and gum tragacanth	Shrimp	Film color change (pink to pale yellow) with increased TVBN after 48 h at 20 °C	Thakur <i>et al.</i> ¹¹⁰
Citric acid	Nanocellulose and PVA	Chinese grass carp	It prevents the generation of TVBN by hindering the microbial and enzymatic growth for 9 days at 4 °C	Wang <i>et al.</i> ²³⁸
Thyme essential oil and shikonin	Chitosan	Tilapia filets	Under 4 °C, TVBN, water loss, and oxidative degradation became lowest for 10 days	Shi <i>et al.</i> ²³⁹
Geraniol	Soy lipophilic protein	Salmon	Overall appearance and color did not show any significant changes at 4 °C for 6 days. Additionally, it able to retains freshness and <i>Pseudomonas</i> growth rate up to 9 days	Sun <i>et al.</i> ²⁴⁰
Lemon peel oil	Starch, pullulan, and glycerol	Salmon	After 5 days at 4 °C, overall appearance was higher than control. Meanwhile, weight loss, and pH values were lowest	Zou <i>et al.</i> ²⁴¹
αS ₂ -casein _{182–207} and αS ₂ -casein _{151–181} peptide	Gelatin and acetic acid	Kashar cheese	Under 4 °C, it hinders the growth of gram-positive, yeast, mold, and aerobic mesophilic bacteria for 21 days	Doğan <i>et al.</i> ²⁴²
Rosemary oil	Whey protein	Cheese	No observable changes in yeasts and molds were identified after 28 days in packed at 4 °C	Zhang <i>et al.</i> ²⁴³
Carvacrol and thymol	Chitosan	Karish cheese	Color and flavor attributes were almost similar to control till 5 days (4 °C)	Gadallah <i>et al.</i> ²⁴⁴
Anethum graveolens oil	Thermoplastic biopolymers	Brie cheese	Under 5 °C, it prevents the growth of <i>Salmonella typhimurium</i> and <i>Listeria monocytogenes</i> for 7 days	Di Matteo <i>et al.</i> ²⁴⁵
Carvacrol	Gellan gum and pineapple nanocellulose	Bread	No visual changes and fungal growth at room temperature for 14 days	Sripahco <i>et al.</i> ²⁴⁶
	Aluminosilicates	Crisps	Lipid oxidation rate was very less up to 30 days at 60 °C	Wrona <i>et al.</i> ²⁴⁷

ultraviolet absorption, making them promising candidates for active food packaging applications.²⁰⁵

5.5 Photothermal and nanomaterial-enhanced packaging

Complementary photothermal films may use nanomaterials such as gold nanoparticles or MXenes to absorb light and convert it into localized heat, disrupting microbial membranes and enhancing antimicrobial efficacy.²¹⁰ Wang *et al.*²¹¹ developed an antibacterial composite by incorporating black phosphorus quantum dots (BPQDs) into a tannic acid-functionalized zeolitic imidazolate framework (ZIF-8-TA). This system showed synergistic antimicrobial effects *via* photothermal ablation (808 nm NIR, 1.5 W cm⁻²), photodynamic ROS generation, and polyphenol-mediated membrane disruption. When added to chitosan films, it preserved grapes by retaining 92.3% ascorbic acid and limiting weight loss to under 5% over 14 days at 4 °C. Compared with conventional packaging, beef has a 3.8 log CFU g⁻¹ lower microbial count and extends shelf-life to 18 days. It inactivated 89.7% of the *Escherichia coli* and 93.2% of the *Staphylococcus aureus* within 2 hours of NIR exposure.²¹¹

5.6 Commercial biobased packaging

While numerous startups and larger enterprises are advancing biobased plastics to compete with traditional petroleum-derived materials, many commercial biopackaging products currently lack active antimicrobial components. Examples of commercial antimicrobial films include Zeomic™ and Microgarde™.²¹² Companies such as TIPA® manufacture compostable films containing between 20% and 80% biobased content that are fully compostable. These films perform well under freezing conditions but degrade when exposed to heat or high-liquid-content foods, limiting their applicability.²¹³ Polyhydroxybutyrate (PHB), a bacterial biopolymer from the polyhydroxyalkanoate family, represents a promising biodegradable, biocompatible, and nontoxic alternative. Its microbial synthesis avoids competition with food crops, supporting sustainable packaging material production.²¹⁴ Cellulose derivatives such as carboxymethylcellulose (CMC) are incorporated into composite films with curcumin and zinc oxide nanoparticles to generate packaging materials with enhanced antimicrobial and water vapor barrier properties; however, the intrinsic hydrophilicity of cellulose sometimes limits its practical application.²¹⁵ Edible packaging films blending alginate, aloe vera, and garlic oil have been reported to provide strong barriers and antimicrobial functionalities, highlighting the potential for active packaging applications.²¹⁶ The addition of garlic extract and titanium dioxide nanoparticles to CMC/Arabic gum/gelatin composite films improved their mechanical properties, water vapor and oxygen permeability, and antimicrobial efficacy.²¹⁷

5.7 Intelligent packaging and spoilage detection

In intelligent packaging, nanomaterials such as quantum dots have been exploited to detect bacterial growth through fluorescence signals. Cadmium selenide/zinc sulfide quantum dots exhibit blue fluorescence upon microbial binding, indicating rapid contamination detection. Upconversion fluorescence

systems detect important biomolecules, including proteins, enzymes, and bacteria, enabling the development of test kits for pathogens such as *Escherichia coli*.²¹⁸ Other microbial spoilage indicators include pH-sensitive chitosan-based films that change color upon contamination: chitosan films containing dyes from *Bauhinia blakeana* turn from brown to green upon pork spoilage,²¹⁹ whereas anthocyanins from red cabbage incorporated into chitosan exhibit color variation, indicating milk spoilage.²²⁰ Bacterial cellulose–polypyrrole–zinc oxide nanoparticle films can be used to assess both pH and spoilage in chicken meat, and polyvinyl alcohol–gelatin films loaded with *Amaranthus* leaf extract exhibit colorimetric changes due to the use of betalain pigments for fish and poultry freshness detection.²²¹ An ammonia-sensitive film composed of polyvinyl alcohol, quaternary ammonium chitosan, and cactus pear extract has demonstrated real-time shrimp freshness detection through distinct color shifts from pink to yellow, concurrently providing antioxidant and antimicrobial effects.²²²

Recent advances include pectin-based antimicrobial hydrogels infused with cinnamon essential oil, which absorb moisture within bread packaging and release the oil to suppress mold growth.²²³ Additionally, enzyme-responsive nanofiber membranes that release thymol in response to the presence of pectinase have been developed to inhibit *Aspergillus niger* in citrus fruits.²²⁴ Colorimetric analysis and fluorescence spectroscopy are commonly used for evaluating film responsiveness. Crosslinking caseinate films with glyoxal produces insoluble networks capable of gradual enzymatic release and sustained antimicrobial activity against pathogens such as *E. coli* and *S. aureus*. Gallic acid coatings enhance these effects, demonstrating potential for food packaging applications requiring prolonged microbial inhibition.²²⁵

6. Applications across food products

Biopolymers and natural extract-based antimicrobial packaging films have been explored for various types of food products (Table 4).

6.1 Fresh produce

To preserve fresh-cut jackfruit, Wei *et al.*²²⁶ incorporated a zein–magnolol mixture (0.5 v/v) into a sodium carboxymethyl cellulose film, which can hinder color change and visible mold growth until 8 days at 25 °C. The hydrophobic nature of zein provides a moisture barrier, while magnolol's phenolic chemistry contributes to its antioxidant and antimicrobial activities. This dual functionality minimized weight loss (22.65% *vs.* 32.51% in unpacked samples), delayed lipid peroxidation (lowest malondialdehyde content), and preserved sensory attributes such as flavor and texture. Li *et al.*²²⁷ developed an egg tray-shaped aerogel using gelatin and genipin-crosslinked chitosan, which preserved strawberry quality for five days without visible mycelia. The protein–polysaccharide matrix enhanced firmness and soluble solids retention by reducing water vapor permeability, while cytotoxicity assays confirmed its biocompatibility (93% cell viability at 625 µg mL⁻¹). This demonstrates



how food matrix chemistry (high water activity in strawberries) requires packaging with strong moisture control and antimicrobial release. Han *et al.*²²⁸ added chlorogenic acid (0.3 mg mL⁻¹) to agar films, reporting superior antimicrobial ability. The phenolic hydroxyl groups of chlorogenic acid disrupted the microbial membranes, extending the cherry shelf time to 9 days at 25 °C with 75% RH and reducing spoilage to 12.5%. Sensory evaluation confirmed an acceptable taste and texture, linking chemical stabilization directly to consumer acceptability. de Freitas *et al.*²²⁹ incorporated grape stalk phenolics (6% wt.) into polylactic acid films, lowering the peroxide index of sunflower oil after 30 days. Here, the antioxidant-rich phenolic chemistry reduced oxidative rancidity, revealing how packaging can be tailored to lipid-rich matrices. Ahn *et al.*²³⁰ introduced lemon-grass oil into polyvinyl alcohol films for gimnap storage at 25 °C and 90% RH. Compared with that in the packed samples, the microbial growth in the unpacked samples increased to 6.8 log CFU mL⁻¹ after 12 h, whereas it was 4.2 log CFU mL⁻¹ in the packed samples. However, rapid release (~60% within 24 h) led to diminished antimicrobial efficacy, highlighting the need for controlled release systems to match the high-moisture, high-pH food matrix of gimnap. Xu *et al.*²³¹ added chitosan/ZIF-67-Cu powders (0.5–2%) into carboxymethyl cellulose films for cherry tomatoes. The hybrid system provided antimicrobial Cu ion release while maintaining low migration (86.4% cell viability at 0–400 µg mL⁻¹), reducing weight loss to ~4% over 12 days. This finding illustrates how balancing antimicrobial efficacy with toxicological safety is critical in fresh produce applications. Zhu *et al.*²³² reported that compared with unpacked controls (7.01%), oxidized corn starch/polybutylene adipate terephthalate films reduced weight loss in cherry tomatoes (3.69%) and grapes (3.65%). The low water vapor permeability of the starch–polyester blend was directly correlated with reduced dehydration and better sensory firmness. Dang *et al.*²⁴⁸ reported that gallic acid in chitosan/N,N-methylenbisacrylamide films minimized grape weight loss (12.13% vs. 18.73% control) and improved hardness (19.55 N vs. 12.55 N). The dense polymer structure restricted oxygen permeation, linking matrix chemistry to oxidative stability and texture preservation. Ashraf *et al.*²⁴⁹ used zein–nisin nanofillers (15%), pullulan, deep eutectic solvent plasticizers, and carboxymethyl chitosan to coat mangoes stored at 25 °C and 70% RH. The antimicrobial peptide nisin inhibited bacterial growth, whereas zein improved barrier properties, resulting in the highest firmness, polyphenol retention, and lowest weight loss. These findings demonstrate how multifunctional coatings can address microbial spoilage, oxidative degradation, and sensory quality simultaneously.

6.2 Meat and poultry products

In modern-day industrial processing, extending the shelf-life of poultry products and their derivatives with conventional packaging poses severe challenges to preserving their poultry-fresh quality because of their increased susceptibility to microbial spoilage. As a result, several innovative approaches that integrate both biobased and functional materials have been

developed. Ahmad *et al.*²³³ fabricated tripolymeric films integrating gelatin, carboxymethyl cellulose, and chitosan with apple polyphenols (APs) for packaging beef. According to the XRD and FTIR results, AP incorporation improved H-bonding, crystallinity, and film structural stability. Additionally, the antioxidant and antimicrobial capacities substantially increased, as evidenced by the effective inhibition of *E. coli* and *S. aureus*, with a shelf-life extension of beef of up to three days at 4 °C. The films resulted in less water loss and lipid/protein oxidation of the product with higher UV barrier properties (60% reduction in transmittance). Natural extracts and biobased coatings rich in antimicrobial and antioxidant properties have also been investigated to extend the shelf-life of meat. Khoshdouni Farahani *et al.*²⁵⁰ reported that clove extract effectively inhibited lipid oxidation and microbial spoilage in chicken fillets stored at 4 °C. Compared with the control, the 3% clove extract significantly reduced the pH, peroxide value, thiobarbituric acid content, TVN, and microbial count. Moreover, the 3% treatment resulted in the highest sensory score, confirming its potential to prolong the shelf-life of chicken fillets. In contrast, Rupérez *et al.*²³⁴ proposed active sachets containing diacetyl, a lactic acid bacterial metabolite with proven antimicrobial action, entrapped in sodium stearate gels and cyclodextrin nanosponges (CDNS). Here, the volatilization mechanism of diacetyl and CDNS was considered within beef packaging environments (at 4 °C) to control pathogens such as *Salmonella* and spoilage microbiota without direct contact. Interestingly, a 77% inhibition rate against *Salmonella* was reported after 11 days. Moreover, *Pseudomonas* spp. and lactic acid bacteria were more than 90% effective for 11 days. Furthermore, some green approaches, such as citrus peel-derived silver nanoparticle films²⁵¹ and pea protein–corn starch films with green tea polyphenols,²⁵² were found to be effective against major pathogens and lipid oxidation in beef. Another example of active packaging involves a bilayer packaging system consisting of an outer layer of gelatin and LAPONITE® (a synthetic clay) with an inner layer composed of electrospun zein fibers incorporating eugenol.²⁵³ The bilayer packaging proved to be beneficial for pork meat, as the LAPONITE® content improved the mechanical and barrier performance, whereas the zein-eugenol layer provided concentration-dependent antimicrobial and antioxidant activities *via* Fickian diffusion, as inferred from previous studies. A study conducted by Xu *et al.*²³⁵ evaluated the dual antimicrobial action of photodynamic food packaging using gelatin and a dialdehyde nanocellulose matrix loaded with berberine-based nanoparticles and ε-polylysine. Eventually, the composite film exhibited robust mechanical strength, fair hydrophobicity, minimal ultraviolet transmittance, and high transparency (86.57%). The antimicrobial mechanism involved synergistic reactive oxygen species generation and membrane disruption under visible light to increase the bacterial count by more than 6 log CFU in 15 min. This mechanism also maintained the shelf-life of pork with lower TVB-N and TBARS values, delaying spoilage for 10 days at 4 °C. For broad-spectrum antimicrobial activity, pectin–gelatin films with microalgal extracts in deep eutectic solvents were proposed by Karabulut & Goksen²³⁶ for



chicken meat. They reported that the inhibition of *E. coli* and *S. aureus* increased by 80 and 20%, respectively, with the total mesophilic bacteria reducing to $5.59 \log \text{CFU g}^{-1}$ after 7 days. Thus, active packaging renders multifunctional solutions to these concerning constraints involved in poultry processing and preservation.

6.3 Seafood and fishery products

The seafood and fishery processing industries are currently centered around green production lines without any exception to their packaging. The packaging line has thus gradually shifted toward the utilization of natural extracts and biopolymer-based active packaging for these products. As fish and other seafoods are among the most easily spoiled food products, researchers are investigating the antibacterial characteristics of natural resources to replace synthetic chemicals. Several investigations have shown that natural extracts have immense potential to hinder microbial growth and lipid oxidation, the primary constraints to the industrial storage of seafood products. For example, Shi *et al.*²³⁹ developed chitosan films plasticized with citric acid-based deep eutectic agents (CA-DEAs) and optimized the film composition at 0.5% CA-DEA. The film exhibited a tensile strength of 47.98 MPa and improved antibacterial zones for *E. coli* and *S. aureus* (up to 23.6 mm), with strong DPPH and ABTS radical scavenging properties. Particularly for tilapia, it notably reduced water and protein loss, maintained TVB-N below 20 mg/100 g, and extended shelf-life by more than 10 days at 4 °C. Similarly, Babaei *et al.*²⁵⁴ reported that gelatin–sodium alginate composite coatings enriched with phycocyanin effectively delayed the spoilage of whiteleg shrimp during refrigerated storage. Compared with the control conditions, the 10% and 20% phycocyanin treatments significantly reduced bacterial growth, oxidative spoilage, and chemical deterioration. Moreover, the sensory attributes remained stable, highlighting phycocyanin as a promising natural antioxidant additive for extending shrimp shelf-life. In another study, Jeon *et al.*²⁵⁵ incorporated malic acid (MA) into whey protein isolate coatings for steamed fish paste. They then applied an in-package cold plasma treatment to enhance the coating efficacy and observed an increase in the MA diffusion rate of up to 1.5 times. The treatment was effective enough for microbial inhibition of *Salmonella* ($\sim 2.5 \log \text{CFU g}^{-1}$ reduction) and *Listeria monocytogenes* ($\sim 1.8 \log \text{CFU g}^{-1}$) for over 28 days at 4 °C, with stable product color and pH. Wu *et al.*²⁵⁶ also developed multifunctional chitosan–quercetin-based films for green packaging with superior mechanical properties, >90% antimicrobial efficacy, and rapid free radical scavenging (80% in 5 minutes). Additionally, the film provides real-time spoilage monitoring with a 70-day self-degradation limit and has been found to significantly slow TVB-N increases in stored fish. Wang *et al.*²³⁸ designed a self-healing cellulose nanofibril–PVA hydrogel system with curcumin-loaded ZIF-8 to preserve grass carp fillets. It exhibited 193.5% greater tensile strength and DPPH scavenging rates up to 95%. They could track the extension of shelf life up to 9 days with an in-package ammonia-induced color shift, indicating freshness.

Several works have also demonstrated advanced nanotech-based antimicrobial packaging with natural inputs, such as engineered biopolymer films embedded with cinnamaldehyde-loaded chitosan nanoparticles in a chitosan–PVA–fish gelatin matrix²³⁷ or nanofiber films with nanochitin–nicin complexes fabricated *via* coaxial electrospinning.²⁵⁷ The homogeneously dispersed nanoparticles improved the film tensile strength, thermal stability, and UV-barrier properties, along with an inhibition zone of up to 6.6 mm for foodborne bacteria such as *S. aureus*, *L. monocytogenes*, *E. coli*, and *S. enteritidis*. The sustained release of antimicrobials promoted the extension of the shelf-life of rainbow trout fillets to 12 days at 4 °C. Nanotech-based smart packaging is also promising when designed with lyophilic proteins from legumes such as soy coupled with nanoemulsions and hydroxypropyl methylcellulose taken as a matrix.²⁴⁰ The bilayer design with pH-responsive indicators (shikonin) enhanced the water-barrier performance, with a shelf-life extension of up to 9 days for salmon. For salmon preservation, edible starch–pullulan films with geraniol are also effective and can achieve hydrophobicity (contact angle of 68.1°), reduced water solubility (37.6%), and high DPPH/ABTS scavenging (up to 56–66%) with product freshness for up to 7 days (maintaining bacterial counts under $6 \log \text{CFU g}^{-1}$).²⁴¹ As the packaging industry has been encouraged to use environmentally benign natural polymers such as proteins, polysaccharides, and their derivatives, shrimp packaging is also notably reshaped from conventional packaging *via* synthetic plastics. For example, Feng *et al.*²⁵⁸ developed sodium alginate films embedded with cobalt-based metal–organic framework nanoparticles, which achieved a tensile strength of 96.4 MPa, high antibacterial rates of up to 99.9% for *S. aureus* and rapid color changes with ammonia sensitivity, indicating shrimp spoilage. Furthermore, Thakur *et al.*¹¹⁰ demonstrated the antibacterial efficacy of kodo millet starch–gum tragacanth films with beetroot peel extract, which markedly improved the water vapor barrier properties and visual pH sensitivity for shrimp freshness, which was correlated with TVB-N levels.

6.4 Dairy and soy products

To delay the deterioration of tofu, Cui *et al.*²⁵⁹ added nisin (0.312 mg g^{-1}), which effectively inhibited bacillus growth. Nisin is a cationic peptide that interacts with bacterial cell membranes to form pores that disrupt viability. Its incorporation maintained its textural firmness and color stability for 10 days at both 4 °C and 37 °C, demonstrating how peptide chemistry can be modified in high-moisture, protein-rich matrices. In another study, Gao *et al.*²⁶⁰ used dialdehyde cellophane for tofu packaging, preventing color changes for 3 days at room temperature. The aldehyde groups crosslinked with protein structures, stabilizing the surface appearance, although microbial counts and sensory attributes indicated limited long-term efficacy. This underscores the need for controlled release systems in high-pH soy matrices.

Zhang *et al.*²⁴³ extracted peptides ($\alpha\text{s2-casein}_{182-207}$ and $\alpha\text{s2-casein}_{151-181}$) from dairy products and incorporated them into whey protein films. These peptides restricted *E. coli* growth to <1



log CFU g⁻¹ for 21 days and prevented yeast/mold growth for 28 days at 4 °C. The peptide–protein synergy illustrates how food matrix chemistry (casein–whey interactions) can be exploited for targeted antimicrobial release while maintaining sensory neutrality. Essential oils are often unsuitable for dairy due to pungency and volatility. Gadallah *et al.*²⁴⁴ addressed this by encapsulating rosemary oil in chitosan nanoparticles for Karish cheese. This nanocarrier system masked strong odors, stabilized the oil against light degradation, and preserved flavor and color attributes for 28 days. Quantitatively, microbial reductions were dramatic: *A. flavus* (4.97 log), *C. albicans* (6.82 log), and *P. aeruginosa* (7.72 log) within 3 days, with complete elimination by day 15. This highlights how encapsulation connects sensory acceptability with antimicrobial potency. However, Doğan *et al.*²⁴² introduced lemon peel oil into gelatin–acetic acid films to preserve Kashar cheese at 4 °C. The hydrophobic terpenes in lemon oil provided broad-spectrum antimicrobial activity against Gram-positive bacteria, yeasts, and molds, extending the shelf-life to 21 days. However, sensory evaluation highlighted the challenge of balancing antimicrobial efficacy with flavor acceptability, a critical performance metric in dairy systems. Similarly, Di Matteo *et al.*²⁴⁵ introduced carvacrol and thymol into thermoplastic biopolymers, which inhibited *Salmonella typhimurium* and *Listeria monocytogenes* in brie cheese at 5 °C. Carvacrol at an 8% concentration strongly inhibited *Listeria* after 10 days, although *Lactobacillus* remained unaffected, indicating that the selective antimicrobial action preserved the beneficial microflora. However, authors such as Anari *et al.*²⁶¹ modified LDPE films with acrylic acid and natamycin for doogh packaging. This ensured that the yeast counts remained below the Iranian standard limit (87 CFU mL⁻¹ vs. 100 CFU mL⁻¹) after 23 days at 25 °C, confirming regulatory compliance and microbial stability in acidic dairy beverages. Similarly, Bruni *et al.*²⁶² applied PHBV films treated with lauroyl arginate ethyl to spreadable cheese at 4 °C. The antimicrobial agent diffused into cheese at 21.5 mg, within the safety threshold (28.9 mg per body weight), effectively inhibiting *Penicillium roqueforti* and *Micrococcus luteus*. Importantly, migration studies confirmed compliance with intake limits (0.5 mg per kg body weight), linking toxicological safety with functional antimicrobial performance.

6.5 Bakery- and cereal-based products

To extend the shelf-life of bread, Sripahco *et al.*²⁴⁶ incorporated *Anethum graveolens* oil into gellan gum–pineapple nanocellulose films and incubated them for 14 days at room temperature. The hydrophilic nanocellulose provided structural integrity and moisture regulation, while dill oil contributed to antifungal activity. Bread samples showed no visual changes for up to 21 days, confirming that the volatile compounds of the oil effectively suppressed fungal growth without altering sensory attributes such as color or texture. Kim *et al.*²⁶³ evaluated insect protection in instant noodles by mixing rice chaff, vulgare, and *Tanacetum cinerariifolium* with LLDPE films. At the 0.5% concentration, the repellent efficacy was markedly greater against *P. interuptella* (8.12-fold reduction) than against *T.*

castaneum (2.17-fold reduction). This demonstrates how food matrix chemistry (low-moisture, starch-rich noodles) requires packaging with insect-repellent properties rather than microbial control, broadening the functional scope of antimicrobial packaging.

Wrona *et al.*²⁴⁷ added encapsulated carvacrol to aluminosilicate–LDPE films to protect crisps. The phenolic chemistry of carvacrol provided strong antioxidant activity, reducing lipid oxidation rates at a 30% concentration after 30 days at 60 °C. The malondialdehyde (MDA) content was 45% lower than that of the controls, suggesting that chemical stabilization directly affects the sensory preservation of crisp flavors and aromas. Peighambardoust *et al.*²⁶⁴ loaded polypropylene films with sorbic acid for yogurt dough storage under refrigeration. The moisture content remained stable for 20 days, whereas sorbic acid migration (1.4–3.3 mg dm⁻²) was well below the permitted limit (10 mg dm⁻²). At a concentration of 6%, sorbic acid effectively hindered mold growth and preserved sensory quality, demonstrating regulatory compliance and functional efficacy in high-moisture dough matrices. Settler-Ramírez *et al.*²⁶⁵ incorporated casein hydrolysates into polyvinyl alcohol films for homemade paste creams. The peptides exhibited strong antimicrobial activity, extending the shelf-life to 13 days at 4–24 °C. Their protein-derived chemistry provided controlled release, maintaining microbial stability without altering sensory properties, highlighting the compatibility of dairy-derived antimicrobials with starch-rich bakery matrices.

7. Challenges and limitations

Despite the promising potential of antimicrobial active packaging using biopolymers and natural extracts, several challenges restrict their adoption and commercialization. When incorporated into complex biopolymer matrices, natural antimicrobial extracts often exhibit reduced efficacy. Additionally, factors such as pH, moisture content, temperature and food composition can affect the release kinetics and antimicrobial activity of active compounds. For example, essential oils and polyphenols may degrade or volatilize during processing (especially at high temperatures) and storage. Moreover, the interaction between food components and active agents can lead to compound neutralization, which can reduce their effectiveness. Therefore, attaining uniform dispersion and stabilization of incorporated natural extracts remains important. In addition, the phase separation and poor miscibility between hydrophobic compounds such as essential oils and hydrophilic polymers such as starch could result in poor film integrity. Likewise, the mechanical, barrier, and optical properties of the films may be affected by the incorporation of extract/bioactives. Therefore, an optimization of the formulation and processing conditions is needed.

In addition to technical challenges, the regulatory aspects of antimicrobial packaging are complex and depend on the region. In the EU, active substances must comply with EC regulations 1333/2008 and 450/2009, whereas in the US, the FDA classifies them as food additives or GRAS substances under the FFDCA. However, most natural extracts also lack standardized safety



evaluations or approved usage limits. This further complicates existing issues and commercial adoption. Moreover, the lack of knowledge of any extract or compound may increase consumer concerns regarding the safety, allergenicity, and sensory impact of natural extracts. Some natural extracts, particularly those derived from animal or microbial sources, may pose allergenic risks. Essential oils, for example, may impart strong odors or flavors that alter food acceptability. Animal-derived components such as lysozyme or lactoferrin may trigger allergic reactions or raise ethical concerns. Additionally, the long-term effects of chronic exposure to bioactive compounds released from packaging materials remain underexplored. Therefore, research and development priorities must decisively address regulatory compliance through standardized toxicological testing, migration studies, and harmonized global guidelines; cost-effective manufacturing by developing scalable encapsulation, extrusion, and hybrid processing technologies that ensure reproducibility and reduce production costs; and robust performance stability under realistic supply chain conditions, including variable temperature, humidity, and mechanical stress. By focusing on these priorities, future research can bridge the gap between laboratory innovation and industrial commercialization, ensuring that antimicrobial biopolymer packaging evolves into a safe, effective, and market-ready solution.

8. Future perspectives

Several strategies are emerging to overcome the limitations of current antimicrobial active packaging systems. Compared with traditional extraction methods, sustainable extraction methods, including supercritical fluid extraction, ultrasound-assisted extraction, and enzyme-assisted techniques, are gaining significant research attention for isolating bioactive compounds with minimal environmental impact. These methods not only improve yield but also reduce solvent use and preserve compound integrity. Additionally, green solvents and biodegradable carriers are being explored to increase formulation compatibility. Future research must prioritize scalable, eco-efficient extraction methods coupled with stabilization strategies such as encapsulation and nanocarrier systems. These approaches ensure consistent bioactive compound quality, industrial feasibility, and a reduced environmental footprint. Advanced release systems such as nanocarriers, liposomes, and smart-responsive systems (pH- or temperature-triggered release) offer controlled and targeted delivery of antimicrobial agents. The next decade should focus on quantifying release kinetics in real food matrices, optimizing dosage efficiency, and minimizing sensory interference to bridge the gap between laboratory success and commercial adoption. New hybrid encapsulation methods, such as combining spray drying with gel or cross-linking techniques, help make large-scale production easier by improving the stability of active ingredients, reducing material loss, and allowing continuous, cost-effective manufacturing. Future work must decisively address scalability by integrating continuous processing, cost modeling, and industrial pilot trials to validate the commercial readiness

of biopolymer/extract/nanocarrier hybrid systems. Biopolymer-based antimicrobial packaging supports the circular economy by reducing food waste, improving compostability, and reducing dependence on fossil-based plastics. Further advances in recycling, biodegradation, and life cycle assessment must be coupled with clear regulatory frameworks and consumer awareness strategies to ensure market acceptance and alignment with SDG 12 and SDG 14. Overall, the strategic outlook for the next decade lies in engineering and designing smart, multifunctional packaging systems that combine antimicrobial, antioxidant, and sensing features while simultaneously addressing scalability, regulatory clarity, and consumer trust.

9. Conclusions

Antimicrobial active packaging using biopolymers and natural extracts is a promising alternative for sustainable food preservation. Exploiting and optimizing the inherent antimicrobial properties of plant, animal, and microbial derivatives for incorporation in biopolymer-based food packaging systems can address spoilage-related losses. The current state of the field demonstrates strong laboratory-scale evidence of efficacy, but translation to industrial-scale applications remains limited. Challenges such as compound stability, compatibility with the polymer matrix, regulatory barriers, and scalability must be explored and addressed to realize the full potential of biopolymer/natural extract films. Therefore, advances in the fields of encapsulation and controlled release systems are offering better stabilization as well as improving the efficacy of bioactive compounds. Various studies and reports confirm that integrating antimicrobial packaging into circular economy frameworks and food systems can significantly contribute to sustainable goals (SDG 12 and SDG 14). Strategic priorities for the next decade must focus on quantifying antimicrobial performance under real-world supply chain conditions, developing scalable manufacturing technologies, and establishing standardized testing protocols to ensure reproducibility and safety. Clear regulatory guidelines and efforts to increase consumer awareness could play crucial roles in ensuring the market acceptance of these biopolymer/natural extract-based antimicrobial films. Ultimately, sustained interdisciplinary strategies, engineering-driven innovation, and investment in industrial partnerships are crucial for developing safe, effective, and scalable antimicrobial packaging systems that align with environmental sustainability and public health goals.

Author contributions

Shubhajit Sarkhel, Samandeep Kaur, Rahul Das, Aditi Sharma, Ankan Kheto, Debapam Saha: writing – original draft, investigation; formal analysis; Yogesh Kumar: conceptualization, writing – original draft, writing – review & editing, validation, supervision.

Conflicts of interest

The authors declare that there are no conflicts of interest.



Data availability

All findings and supporting data are available through the respective published sources cited in this work.

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