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# Mechanisms, applications and challenges of natural antimicrobials in food system

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ABSTRACT

In response to the advances in biological research and increasing health awareness, the food industry has shown a growing preference for natural alternatives to synthetic preservatives. Although innovative non-thermal preservation technologies have emerged, such as high-pressure processing, pulsed electric fields, cold plasma, and modified atmosphere packaging, synthetic preservatives like nitrites and sulfites remain widely used due to their low cost and high efficacy. However, these compounds are increasingly associated with adverse health and environmental impacts, driving interest toward natural antimicrobial agents derived from plants, microorganisms, and marine organisms. To enhance the performance and stability of these natural antimicrobials, recent strategies have incorporated nanoencapsulation, edible coatings, and active packaging technologies. In parallel, the rising demand for clean-label products with minimal synthetic additives has further accelerated the shift toward natural preservation approaches, potentially reducing both antibiotic resistance and food waste. In comparison with previous review articles, this article exclusively addresses natural antimicrobials with modern delivery technologies, food-related challenges and its sustainability. This focus fills a critical gap by connecting fundamental antimicrobial mechanisms with their real-world applications in food systems. This review comprehensively examines recent progress in the sources, mechanisms of action, and applications of natural antimicrobials in food systems, emphasizing their potential as sustainable replacements for synthetic preservatives in line with modern consumer expectations.

## 1. Introduction

The food preservation techniques have undergone significant transformation from traditional household techniques to advanced, industrial-scale technologies. Traditional food preservation methods such as drying, salting, curing, freezing, and fermentation were widely practiced in early human societies to extend shelf life and maintain food over seasonal gaps. When foods are dried and cured, the moisture needed by microbes is removed and freezing helps to slow down the enzymatic activity of microorganisms by decreasing the temperature

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(Knorr & Augustin, 2023). Through these techniques food kept fresh and increased both its taste and qualities. Although these approaches laid the foundation for modern preservation technologies, they are often insufficient in combating contemporary foodborne pathogens and may fail to preserve the desired sensory and nutritional qualities of food products.

In recent years, advanced preservation technologies such as High-Pressure Processing (HPP), Pulsed Electric Field (PEF) techniques, cold plasma, irradiation and Modified Atmosphere Packaging (MAP) have emerged as effective alternatives to conventional thermal methods in maintaining food safety and quality. These approaches effectively inactivate microorganisms while maintaining the organoleptic and nutritional properties of foods. For example, HPP utilizes hydrostatic pressure to disrupt microbial membranes without heat; PEF delivers short electrical pulses that damage microbial cell walls, particularly in liquid food products; cold plasma inactivates surface microorganisms using ionized gases; and MAP modifies the internal gaseous environment of packaging to inhibit microbial growth and oxidative reactions (Alaguthevar et al., 2024).

Despite these advancements, synthetic preservatives such as nitrites, sulfites, and benzoates remain widely used due to their low cost, wide-spectrum efficacy, and compatibility with diverse food matrices. However, increasing evidence has linked these chemicals to adverse health effects, including oxidative stress, immune dysfunction, and chronic diseases such as asthma, heart disease, cancer, and cardiovascular disorders Additionally, the environmental footprint associated with the production and disposal of synthetic additives has raised further concerns among both regulators and consumers, thereby accelerating the demand for sustainable and environmentally friendly alternatives (Aldabayan, 2025).

Driven by the clean-label movement and increasing consumer demand for healthier and more sustainable food products, the food industry is progressively shifting toward natural alternatives to synthetic preservatives. Natural antimicrobials derived from plants, microbes and marine sources have shown promising antimicrobial activity while being safer for human and the environment (Table 1). Traditional and modern approaches are converging to offer innovative solutions for food preservation that are environmentally friendly and ensure food safety (Obahiagbon & Ogwu, 2024). Increasing attention to natural antimicrobials is being driven by the convergence of market, consumer, and regulatory pressures. From a market standpoint, they align with the clean-label trend and provide opportunities for product differentiation. Consumers increasingly prefer natural and minimally processed foods, which are perceived as safer and healthier than chemically preserved alternatives. At the same time, regulatory agencies are tightening restrictions on synthetic preservatives while promoting the adoption of safe, natural solutions. Collectively, these drivers are stimulating both research investment and industrial application of natural antimicrobial strategies in food preservation (Karnwal & Malik, 2024)). The rise in natural antimicrobials is also linked to consumers' preference for food products with natural ingredients and transparent labeling. Many consumers now pay closer attention to their diets and actively avoid synthetic chemicals. This transition is largely attributed to heightened consumer awareness of diet and health, as well as the desire for minimally processed foods with transparent ingredient lists (Quinto et al., 2019). Approximately 60 % of consumers believe natural ingredients contribute to better health, and nearly one-third routinely check food labels for synthetic additives (Rampalli et al., 2023). Growing concerns regarding the potential adverse health effects and environmental impact of synthetic preservatives have prompted the food industry to explore safer and more sustainable alternatives. Natural antimicrobials derived from plants, microbes, animals, and marine organisms offer promising antimicrobial properties while aligning with consumer expectations (Mesías et al., 2021). Furthermore, incorporating natural antimicrobials into food products or packaging systems can enhance shelf life and reduce food waste, supporting both economic and ecological sustainability. Nevertheless, maintaining desirable sensory attributes and

**Table 1**
Overview of natural antimicrobials: sources, mechanisms, and food applications.

| Class | Key actives | Mechanism of action | Target microbes | Typical dosage in food | Sensory impact threshold | Delivery techniques | Applications | References |
|---|---|---|---|---|---|---|---|---|
| Plant-based antimicrobials | Thymol, carvacrol, eugenol, quercetin, tannins | Disrupt cell membrane, ROS generation, leakage of contents | *S. aureus*, *E. coli*, *P. aeruginosa*, fungi | 0.05–1 % v/v(fruits, meat) | >0.2 % v/v causes strong aroma in meet products | Nanoencapsulation, microencapsulation | Strawberries, raspberries, beef | De Farias et al., 2025 |
| Animal-derived antimicrobials | Lysozyme, chitosan, lactoferrin, lactoperoxidase | Binds membranes, Iron chelation increases permeability, causes leakage, | Gram-positive bacteria like *Listeria*, *S. aureus* | Lysozyme: 100–400 μg/ml; Chitosan: 0.5–1 % w/v | >200 μg/ml of Lysozyme can cause bitterness in cheese. | Coatings, films, immobilization, blends | Cheese, milk, sea food coatingds | Gupta et al., 2021; Demir et al., 2025 |
| Microbial-based antimicrobial | Nisin, pediocin, lactic acid, acetic acid | Form pores in membranes, disrupt osmotic balance | *Listeria*, *C. perfringens*, *S. aureus* | Nisin: 10–50 μg/ml (dairy/meat); Organic acids: 0.1–1 % w/v | >100 μg/ml, of nisin cause bitternesss, >0.5 % of acid gives a sour flavour | Direct addition, fermentates | Cheese, yogurt, cured meats | Silva et al., 2018 |

ensuring efficacy under clean-label formulations remain major challenges. Continued innovation is therefore crucial to balancing safety, quality, and consumer appeal in the development of natural preservative strategies (Karnwal & Malik, 2024).

The review explores recent progress in the development and application of natural antimicrobials for food preservation. It highlights their antimicrobial mechanisms, as well as their integration with innovative packaging and processing technologies. Furthermore, it discusses their potential to contribute to sustainable and consumer-oriented food systems. By addressing current advances and challenges, this review aims to inform future research, technological innovation, and regulatory development to facilitate a broader adoption of natural preservatives in the food industry.

## 2. Methodology

A systematic literature search was conducted across major scientific databases, including PubMed, Scopus, Web of Science, and Google Scholar, covering publications from 2020 to 2025. The search strategy employed combinations of keywords such as "natural antimicrobials," "plant-derived antimicrobials," "bacteriocins," "chitosan," "essential oils," "marine bioactives," "food preservation," and "clean-label." The initial search retrieved approximately 500 articles. After removal of duplicates, studies were screened at the title and abstract level, followed by a full-text evaluation of potentially relevant papers. Inclusion criteria comprised studies reporting (i) mechanisms of action, (ii) extraction procedures, (iii) delivery systems, or (iv) applications of natural antimicrobials in food systems. Exclusion criteria included non-English publications, non-food-related studies, reviews without experimental or clinical evidence, and reports lacking relevance to food applications. Following this screening process, approximately 150 articles were included in the final synthesis.

## 3. Types of natural antimicrobials

### 3.1. Plant-based antimicrobials

#### 3.1.1. Essential oils

Essential oils are volatile, aromatic compounds extracted from herbs, spices, and medicinal plants, and they represent one of the most widely explored classes of natural antimicrobials in food systems (Al-Maqtari et al., 2022). For instance, thyme oil (*Thymus vulgaris*) is considered as an efficient antimicrobial agent due to its high content of thymol (44.6 %), p-cymene (26.6 %), gamma-terpinene (7 %) and carvacrol (6 %) (Ricardo-Rodrigues et al., 2024). Recent studies demonstrated that a mixture of thyme and oregano oils in an 8:2 ratio effectively inhibits the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Meerasri et al., 2024). Incorporation of thyme oil into nanocellulose-based films reduces fungal contamination, thereby enhancing the safety and shelf life of raspberries stored under refrigeration. Active compounds from such packaging gradually migrate into the food matrix, disrupting microbial cell membranes, inhibiting spoilage, and helping preserve sensory quality. Oregano, obtained from the dried leaves of *Origanum vulgare*, rich in thymol and carvacrol, has attracted interest for its antimicrobial properties in food preservation (Bukovská et al., 2007). Incorporation of oregano oil into chitosan- or nanocellulose-based coatings effectively reduces microbial growth and biofilm formation on food surfaces (De Farias et al., 2025). It is reported that *soy protein films* incorporating *3 % oregano oil* effectively *inhibit* the *growth of Escherichia coli*, *Listeria monocytogenes*, *and Staphylococcus aureus on raw beef, without adversely affecting taste or texture* (Yemiş & Candoğan, 2017). Clove plants, such as *Eugenia caryophyllata* and *Syzygium aromaticum*, are primary sources of eugenol, which constitutes approximately 85 % of clove oil and exhibits strong antimicrobial and antioxidant activities (Silva et al., 2024). The clove leaf oil has been demonstrated to protect fruits such as berries and grapes from UV radiation and fungus growth, thereby extending shelf life (Hasheminejad et al., 2019).

These three essential oils exert their antimicrobial effects primarily through the gradual release of phenolic compounds into the food matrix. These phenolic compounds disrupt microbial cell membrane, causing leakage of vital intracellular constituents and ultimately resulting in cell death. Furthermore, they inhibit biofilm formation, thereby reducing microbial resistance and enhancing food preservation. Antioxidants from essential oils protect food further protect food products from oxidative degradation. Incorporation of essential oils into polymeric packaging materials allows for controlled and sustained release of antimicrobial agents during storage. Combining multiple essential oils within food packaging films has demonstrated enhanced antimicrobial efficacy at reduced concentrations (Ricardo-Rodrigues et al., 2024).

#### 3.1.2. Phenolic compounds

Phenolic compounds, a diverse group of secondary metabolites naturally present in plants, have gained considerable attention in food science due to their dual role as antimicrobial and antioxidant agents, making them highly valuable for applications in food preservation (De Rossi et al., 2025). Phenolic acids are widely distributed in edible plants and known for their antimicrobial and antioxidant properties. Among them, ferulic acid, *p*-coumaric acid, and caffeic acid are the most commonly (Babaei-Rad et al., 2025). These compounds exert antimicrobial effects by disrupting bacterial cell membranes and inhibiting key enzymes involved in energy metabolism. In addition, phenolic acids mitigate oxidative degradation in food products. Biodegradable polyhydroxybutyrate-co-valerate (PHBV) films incorporated with ferulic and p-coumaric acids have demonstrated enhanced resistance to microbial contamination and oxidative stress. This incorporation contributes to prolonged shelf life by preventing microbial spoilage and lipid oxidation. Furthermore, phenolic acids incorporation improves the mechanical and barrier properties of packaging materials, making them suitable for advanced food packaging applications (Ordoñez et al., 2022; Venezia et al., 2024).

Flavonoids and tannins are two major subclasses of phenolic compounds, recognized for their broad-spectrum antimicrobial activities (Roy et al., 2022). Flavonoids such as quercetin, kaempferol and myricetin work exert antimicrobial effects by disrupting bacterial cell membranes and inhibiting biofilm formation, hereby reducing the risk of long-term food contamination. To achieve both antibacterial and antioxidant effects, edible coatings and films are frequently formulated using plant extracts rich in flavonoids (Lopes et al., 2023). By inhibiting biofilm development, these compounds enhance food safety by limiting microbial proliferation and spoilage (Faleye et al., 2023). Tannins, on the other hand naturally found in grape skin, pomegranate and the bark of various tree species (Fraga-Corral et al., 2021). They exhibit antimicrobial activity through reactive oxygen species (ROS) scavenging and by offering UV protective properties. Recent studies have focused on incorporating tannins into PHBV films to enhance their functional properties for food packaging applications. The robust mechanical integrity of tannin-based films makes them suitable for protecting food products during storage and distribution (Santulli et al., 2025).

#### 3.1.3. Alkaloids and terpenoids

Alkaloids and terpenoids, two structurally diverse groups of plant-derived secondary metabolites, have emerged as important natural antimicrobials owing to their broad spectrum of bioactivity, unique mechanisms of action, and potential applications in food preservation and active packaging (Deshmukh & Gaikwad, 2024). Medicinal plants such as *Moringa oleifera*, *Rauwolfia serpentina* and *Catharanthus roseus* are recognized as rich sources of bioactive alkaloids with demonstrated antimicrobial potential (S. Kumar & Singh, 2023). Furthermore, isoprenoids (also known as terpenoids) represent another major class of antimicrobial compounds. These molecules are typically categorized into monoterpenes (e.g., thymol, carvacrol, α-terpineol), sesquiterpenes,

and diterpenes, which are predominantly derived from essential oils of plants including *Origanum vulgare* (oregano), *Thymus vulgaris* (thyme), *Eucalyptus* spp., and *Rosmarinus officinalis* (rosemary) (Deshmukh & Gaikwad, 2024).

Alkaloids and terpenoids exert their antimicrobial effects primarily through disruption of bacterial cell membranes. Lipophilic alkaloids and terpenoids interact with the lipid bilayer, increasing membrane permeability and fluidity, which leads to the leakage of ions and cytoplasmic contents, disruption of proton gradients, and subsequent cell death. Additionally, alkaloids interfere with bacterial gene expression, inhibiting the biosynthesis of virulence factors and the functionality of secretion systems. Both alkaloids and terpenoids are known to prevent biofilm formation, thereby enhancing microbial susceptibility to antimicrobial agents during food storage. Terpenoids also induce oxidative stress in microbial cells through the generation of ROS, leading to further cellular damage. In addition, both alkaloids and terpenoids can inhibit bacterial efflux pumps, thus enhancing the intracellular accumulation and efficacy of antimicrobial agents (Generalić Mekinić & Šimat, 2025). These properties make alkaloids and terpenoids attractive candidates for integration into food packaging and edible coatings aimed at extending shelf life and enhancing microbial safety (Kumar, Ramakanth, et al., 2022). Advances in polymer blending and nanoencapsulation have further improved their stability, controlled release, and overall efficacy in food systems. Notably, alkaloid-rich plants are being explored as natural alternatives to synthetic preservatives due to their ability to inhibit common foodborne pathogens such as *E. coli*, *S. aureus*, and *C. albicans*, thereby contributing to the prevention of food spoilage and contaminations (Generalić Mekinić & Šimat, 2025).

Plant-based antimicrobials are widely accepted due to their antioxidant properties and broad-spectrum activity against bacteria, fungi, and yeast. However, their strong flavors and aromas—particularly from phenolic compounds, can negatively impact the sensory quality of foods. In addition, they are sensitive to light and temperature, requiring encapsulation or protective delivery systems to maintain stability. While such technologies improve efficacy, they also increase production costs, which limits large-scale application in food preservation (Hassid et al., 2025).

### 3.2. Animal-based antimicrobials

#### 3.2.1. Lysozyme

Among animal-derived antimicrobials, lysozyme is one of the most extensively studied enzymes due to its natural abundance, broad-spectrum activity against various bacteria, and versatile applications in food systems (Khorshidian et al., 2022). Lysozyme is an antimicrobial enzyme capable of hydrolyzing the β (1 → 4) glycosidic bonds between *N*-acetylmuramic acid (NAM) and *N*-acetylglucosamine (NAG) in the peptidoglycan layer, ultimately leading to cell lysis and the release of intracellular contents (Gupta et al., 2021). It has been incorporated into edible films made of biopolymers such as whey protein, chitosan, and cellulose nanofibers. Recent studies have explored the immobilization of lysozyme onto biomaterials through layer-by-layer (LbL) deposition techniques, which enhance their antimicrobial efficacy. In addition to the antimicrobial function, lysozyme-based coatings can reduce moisture transmission, thereby improving the shelf life and safety of food products (Hasan et al., 2025).

#### 3.2.2. Chitosan and chitosan derivatives

Chitosan, a polycationic polysaccharide primarily derived from the exoskeletons of marine crustaceans such as crabs and shrimps, exhibits potent antimicrobial activity (Chandarana et al., 2025). Chitosan interacts electrostatically with negatively charged microbial cell membranes, compromising membrane integrity and increasing permeability. This disruption facilitates the leakage of intracellular components, ultimately resulting in microbial growth inhibition (Enejiyon et al., 2025). While some polysaccharides extracted from seaweed (e.g., alginate, carrageenan) are also used in edible coatings, chitosan remains a key bioactive agent due to its functional versatility (Chandarana et al., 2025).

Chitosan and its derivatives are extensively employed in edible films and coatings for perishable foods such as fruits, vegetables, seafood, and meats, where they effectively extend shelf life by minimizing microbial spoilage and slowing enzymatic and oxidative degradation (Wang et al., 2017). The incorporation of natural additives such as beetroot extract, curcumin, and garlic enhances the antioxidant and antimicrobial activities of chitosan-based coatings, contributing to improved food preservation and, in some cases, enhanced sensory attributes. Furthermore, chitosan coatings improve moisture barrier properties and mechanical strength, thereby enhancing packaging performance.

To overcome inherent limitations such as low solubility and reduced mechanical integrity under acidic conditions, various approaches including nanocomposite integration and polymer grafting have been explored. Owing to its biodegradability, cost-effectiveness, and regulatory approval, chitosan is regarded as a promising material for sustainable and functional food packaging systems (El Alami El Hassani et al., 2024).

#### 3.2.3. Lactoferrin & lactoperoxidase

Milk contains lactoferrin, a natural iron-binding glycoprotein that sequesters free iron, thereby depriving microbes of an essential growth factor and compromising their membrane integrity (Demir et al., 2025). Additionally, lactoferrin inhibits biofilm formation by downregulating the expression of pathogen-associated virulence factors. When incorporated into biopolymer-based food packaging, lactoferrin significantly enhances the mechanical strength, barrier properties, and antioxidant and antimicrobial activities of the films, thereby extending food shelf life and ensuring microbiological safety (Duda-Chodak et al., 2023). Similarly, lactoperoxidase (LPO), a heme-containing enzyme, catalyzes the reaction between hydrogen peroxide and thiocyanate to produce hypothiocyanite ($OSCN^-$) and hypothiocyanous acid (HOSCN). These reactive compounds oxidize sulfhydryl groups in microbial enzymes and membrane proteins, resulting in membrane disruption and inhibition of essential metabolic functions (Bafort et al., 2014). LPO has been widely applied in preserving the sensory quality and microbial stability of various food products (Yousefi et al., 2022).

Animal-based antimicrobials are generally recognized as safe (GRAS) and have demonstrated effectiveness in the preservation of dairy, meat, and seafood products. Lysozyme, for example, is effective in these matrices, but its antimicrobial spectrum is narrow, primarily targeting Gram-positive bacteria (Hasan et al., 2025). Regulatory acceptance has facilitated their use in certain food categories, but broader application remains constrained by this limited spectrum.

### 3.3. Microbial derived antimicrobials

#### 3.3.1. Bacteriocins

Bacteriocins are defined as proteinaceous toxins produced by certain bacteria to inhibit the growth of pathogenic organisms. Among them, nisin and pediocin produced by *Lactococcus lactis* and *Pediococcus* species, respectively are widely studied due to their effectiveness against numerous foodborne pathogens, including *L. monocytogenes*, *Clostridium perfringens* and *Staphylococcus aureus* (Khorshidian et al., 2022). They exert the antimicrobial effect by binding to bacterial membranes and forming transmembrane pores, which compromise the osmotic balance and lead to cell lysis and death. In the dairy industry, nisin is commonly used as a natural preservative, especially in cheese production, to inhibit spoilage and extend shelf life. Pediocin exhibits broad-spectrum antimicrobial activity, with stability across a wide range of temperatures and pH conditions, making it particularly effective in controlling *L. monocytogenes* in meat products (Silva et al., 2018). Bacteriocins, especially nisin and pediocin are considered to be the most commercially successful natural antimicrobial due to their role in food

preservation and its stability under the processing temperatures (Surati, 2020).

### 3.3.2. Organic acids

Organic acids and their derivatives, such as lactic acid and acetic acid, are naturally produced during microbial fermentation and are widely used as food preservatives. Their antimicrobial activity primarily stems from their ability to lower the pH of the food matrix and penetrate microbial cell membranes in their undissociated form. Once inside the cell's cytoplasm, organic acids dissociate, leading to an intracellular pH increase and disrupting critical metabolic processes, including enzyme activity and ATP synthesis, thereby inhibiting microbial proliferation or inducing cell death. As a result, organic acids are commonly incorporated into a wide range of food products to enhance microbiological safety, maintain freshness, and extend shelf life (Sorathiya et al., 2025; Wang et al., 2024).

### 3.3.3. Natural antimicrobials from seaweed and algae

Natural polyphenols and polysaccharides derived from seaweed and algae, including phlorotannins and fucoidans, exhibit potent antimicrobial activity against spoilage organisms and foodborne pathogens. Brown seaweeds contain phlorotannins that disrupt bacterial and fungal cell membranes, inhibit ergosterol biosynthesis essential for fungal growth, and effectively control pathogens such as *L. monocytogenes* and *E. coli* (Kumar, Ramakanth, et al., 2022). These compounds not only prevent microbial adhesion and biofilm formation, thereby maintaining food quality, but also provide antioxidant properties that contribute to food preservation (Eom et al., 2012).

Seaweed extracts have been increasingly applied in food packaging, where gel-forming polysaccharides such as alginate and carrageenan are combined with natural preservatives or plant extracts to produce biodegradable, edible films that inhibit microbial proliferation on food surfaces (Perera et al., 2021). For instance, seaweed-based films incorporated with neem leaf extract demonstrate strong antimicrobial efficacy against a broad spectrum of bacteria while maintaining high flexibility, making them promising candidates for eco-friendly packaging solutions. These films not only extend product shelf life by preventing microbial spoilage but also reduce reliance on conventional plastic packaging (Perera et al., 2021). An overview of natural antimicrobials from various sources is illustrated in Fig. 1.

Microbial-based antimicrobials, particularly bacteriocins such as nisin and pediocin, are considered the most commercially successful natural antimicrobials. They are stable under a wide range of processing temperatures and have been approved for use in multiple food systems, making them closest to widespread commercialization (Surati, 2020). Beyond bacteriocins, other microbial-derived antimicrobials exhibit high potency at low concentrations and show stability under processing conditions, but many suffer from narrow antimicrobial spectra, which restricts their broader use (Liu et al., 2020). The relative strengths and limitations of different classes of natural antimicrobials are summarized in Table 2. In general, microbial-based antimicrobials (particularly nisin) are the most advanced in terms of commercialization, while plant- and animal-based compounds face greater challenges related to sensory effects, stability, spectrum of activity, and cost-effectiveness.

## 4. Mechanism of action of natural antimicrobials

Natural antimicrobials exert their inhibitory effects on foodborne pathogens through multiple interconnected mechanisms that target microbial structures, metabolic enzymes, genetic systems, and regulatory pathways. These include cell wall and membrane disruption, enzyme inhibition, energy depletion, oxidative stress induction, and interference with genetic functions.

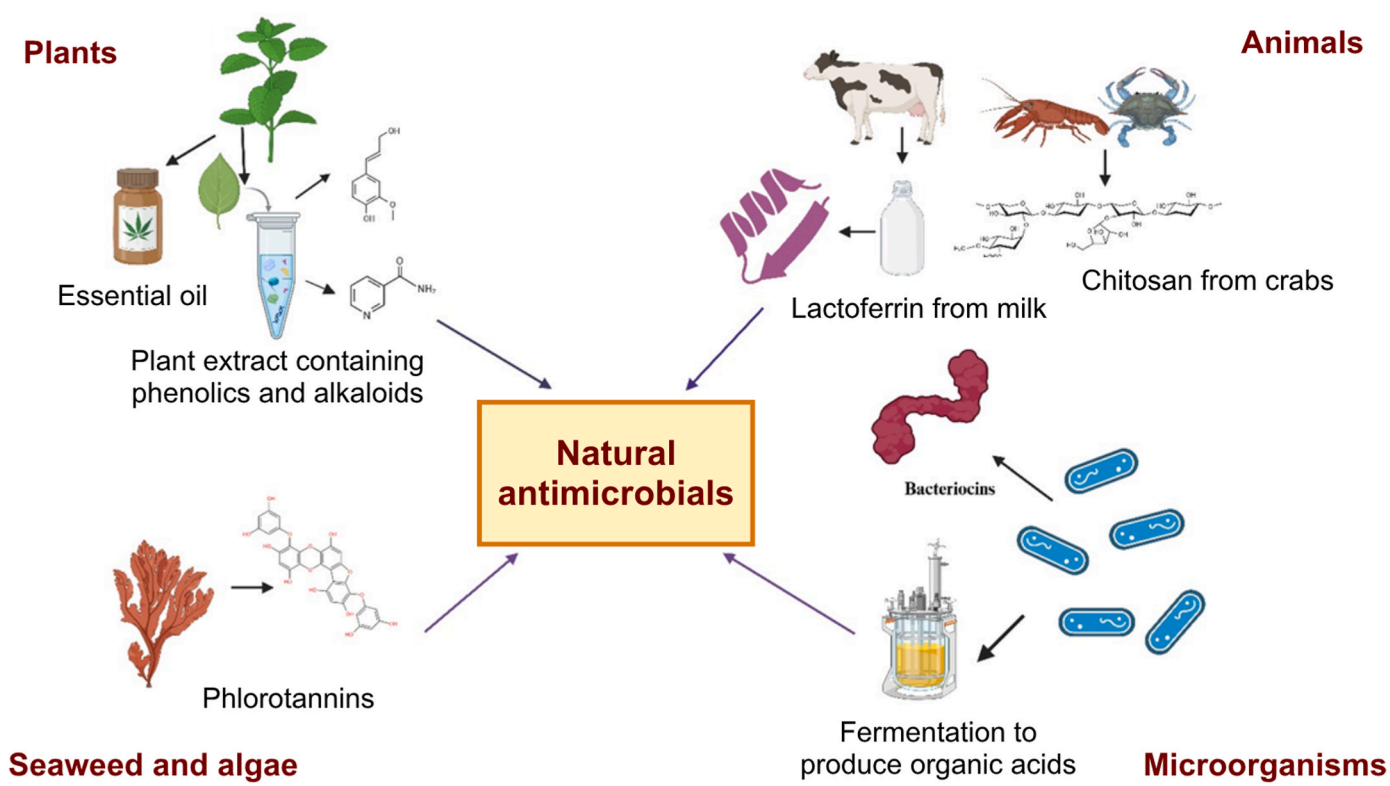


**Fig. 1.** Representative sources of natural antimicrobials. This figure summarizes major sources of natural antimicrobials used in foods: plants (essential oils and extracts rich in phenolics/alkaloids), animals (milk proteins such as lactoferrin; chitosan from crabs), seaweeds and algae (phlorotannins from brown algae), and microorganisms (bacteriocins such as nisin/pediocin; fermentation-derived organic acids).

**Table 2**
A comparative summary table for different types of antimicrobials.

| Antimicrobial type | Active compound (s) | Food applications | Limitations | References |
|---|---|---|---|---|
| Plant-based antimicrobials | Thymol, carvacrol, eugenol, quercetin, tannins | Fruits, vegetables, meat, baked goods | Strong aroma/flavour and instable | Silva et al., 2024 |
| Animal-derived antimicrobials | Lysozyme, chitosan, lactoferrin, lactoperoxidase | Dairy, meat and seafood coatings | Limited spectrum(mostly against gram positive bacteria), allergenicity and solubility issues | Gupta et al., 2021; Demir et al., 2025; Yousefi et al., 2022 |
| Microbial-based antimicrobial | Nisin, pediocin, lactic acid, acetic acid | Dairy, meat and beverages | Limited spectrum(mostly against gram positive bacteria), pH sensitive | Sorathiya et al., 2025; Wang et al., 2024 |

### 4.1. Cell wall and membrane disruption

Chitosan and essential oils exert antimicrobial effects primarily through the disruption of microbial cell membranes and interference with vital cellular functions. Due to its polycationic nature, chitosan interacts electrostatically with negatively charged microbial cell surfaces, increasing membrane permeability and ultimately causing leakage of intracellular components and cell lysis (Ardean et al., 2021). Chitosan nanoparticles, particularly when combined with thyme essential oil, have demonstrated enhanced antimicrobial activity by significantly compromising the membrane integrity of both *E. coli* and *S. aureus* cells. Similarly, c chitosan-based films have been reported to inhibit *Listeria* growth targeting and disrupting key peptidoglycan substructures of bacterial cell walls (Mei et al., 2020).

Essential oil constituents such as thymol, carvacrol, and eugenol, due to their hydrophobic nature, are capable of penetrating microbial membranes, thereby increasing permeability and causing leakage of intracellular ions, depletion of ATP, and loss of membrane potential. In addition, these compounds can induce the generation of ROS, which further exacerbate oxidative stress and damage to intracellular targets. Specifically, thymol and carvacrol from thyme and oregano oils simultaneously disrupt both the outer and inner membranes of *L. monocytogenes*, leading to a loss of membrane potential and leakage of ATP and potassium ion (Kachur & Suntres, 2020).

The combination of chitosan and essential oils exhibits synergistic antimicrobial effects, showing enhanced inhibition of pathogen growth compared to their individual applications. This synergy not only enhances membrane disruption and oxidative damage but also impairs critical metabolic functions such as DNA replication and protein synthesis, thereby potentiating their antimicrobial activity (Haghighi et al., 2019).

### 4.2. Enzyme inhibition and energy disruption

Beyond structural damage, many natural antimicrobials target microbial metabolism at the enzymatic level. Several phytochemicals inhibit critical metabolic enzymes essential for microbial survival. For instance, compounds that inhibit lanosterol 14α-demethylase, a key enzyme in ergosterol biosynthesis, compromise fungal cell membrane integrity and suppress growth (Song et al., 2025). Curcumin, a polyphenolic compound, has been shown to inhibit sortase A in *S. aureus*, a crucial enzyme for adhesion and biofilm formation, thereby reducing colonization and persistence. Other phytochemicals impair carbohydrate metabolism and biosynthetic pathways essential to microbial proliferation (Stan et al., 2021).

Another important antimicrobial action is the disruption of ATP production. Some natural antimicrobials directly inhibit ATP synthase or interfere with proton translocation, leading to a significant reduction in cellular ATP levels. For instance, Venturicidin A, binds to specific subunits of ATP synthase, obstructing proton flow and depleting ATP, which results in energy failure and oxidative damage (Yarlagadda et al., 2020). Moreover, ATP itself, when produced or released extracellularly, can chelate iron, limiting its availability for microbial enzymatic processes and thereby further inhibiting metabolism (Tatano et al., 2015). Furthermore, exposure to natural antimicrobials often induces the generation of ROS within microbial cells, which oxidizes proteins, lipids, and nucleic acids, leading to irreversible damage to enzymes and key metabolic pathways. The resulting oxidative stress, when combined with metabolic inhibition, significantly enhances the antimicrobial efficacy of natural compounds (Stan et al., 2021).

### 4.3. Genetic and cellular responses of microbes

Natural antimicrobials also interfere with genetic regulation and induce cellular stress responses. Microbial populations are significantly reduced due to the activation of oxidative stress, DNA damage, and altered gene expression (Papadochristopoulos et al., 2021). Many plant-derived polyphenols, essential oils, bacteriocins, and microbial metabolites stimulate intracellular reactive oxygen species (ROS) production, triggering oxidative stress. Elevated ROS levels exhaust the cell's antioxidant defense and damage critical biomolecules, including lipids, proteins, and nucleic acids. This results in enzymatic inactivation and metabolic dysfunction, impairing replication and homeostasis (Guo et al., 2023; Karnwal & Malik, 2024; Rodrigo & Palop, 2021).

Natural antimicrobials can induce multiple forms of DNA damage, such as strand breaks, base modifications, and cross-linking, which interfere with cellular processes such as gene transcription and replication. Natural products such as genotoxic molecules are able to join certain genotoxic natural compounds act as DNA intercalating agents, hindering DNA and RNA synthesis and subsequently disrupting transcriptional and translational processes essential for microbial survival. Bacteriocins produced by *Lactobacilli* species, when incorporated into food packaging systems, have shown efficacy in inhibiting biofilm formation and suppressing virulence factor expression in various foodborne pathogens (Imade et al., 2021). Natural antimicrobials can interfere with bacterial regulatory networks, altering the synthesis of enzymes, toxins, and other essential metabolites. Studies have shown that plant extracts and essential oils disrupt quorum sensing pathways in *Pseudomonas* and *Staphylococcus* spp., thereby limiting biofilm formation and enhancing susceptibility to antimicrobial agents ( Kodaparthi et al., 2024).

Biodegradable polymer matrices, edible coatings, and films are increasingly used as delivery systems for these antimicrobial agents, enabling their sustained and controlled release onto food surfaces (Kumar et al., 2021). These multifunctional mechanisms provide a comprehensive defense against microbial contamination and contribute to the development of advanced antimicrobial packaging systems. As illustrated in Fig. 2, these antimicrobial strategies—including membrane disruption, enzyme inhibition, metabolic interference, and genetic damage—collectively contribute to the effective control of foodborne pathogens.

## 5. Application of natural antimicrobials

Natural antimicrobials have been widely used in the food industry to enhance food safety, prolong shelf life, and meet the increasing consumer demand for eco-friendly and clean-label products. Extracts derived from plants, microorganisms, and other natural extracts are frequently incorporated into edible coatings, essential oils, and bioactive films for the packaging fruits, vegetables, and other perishable (Yousuf

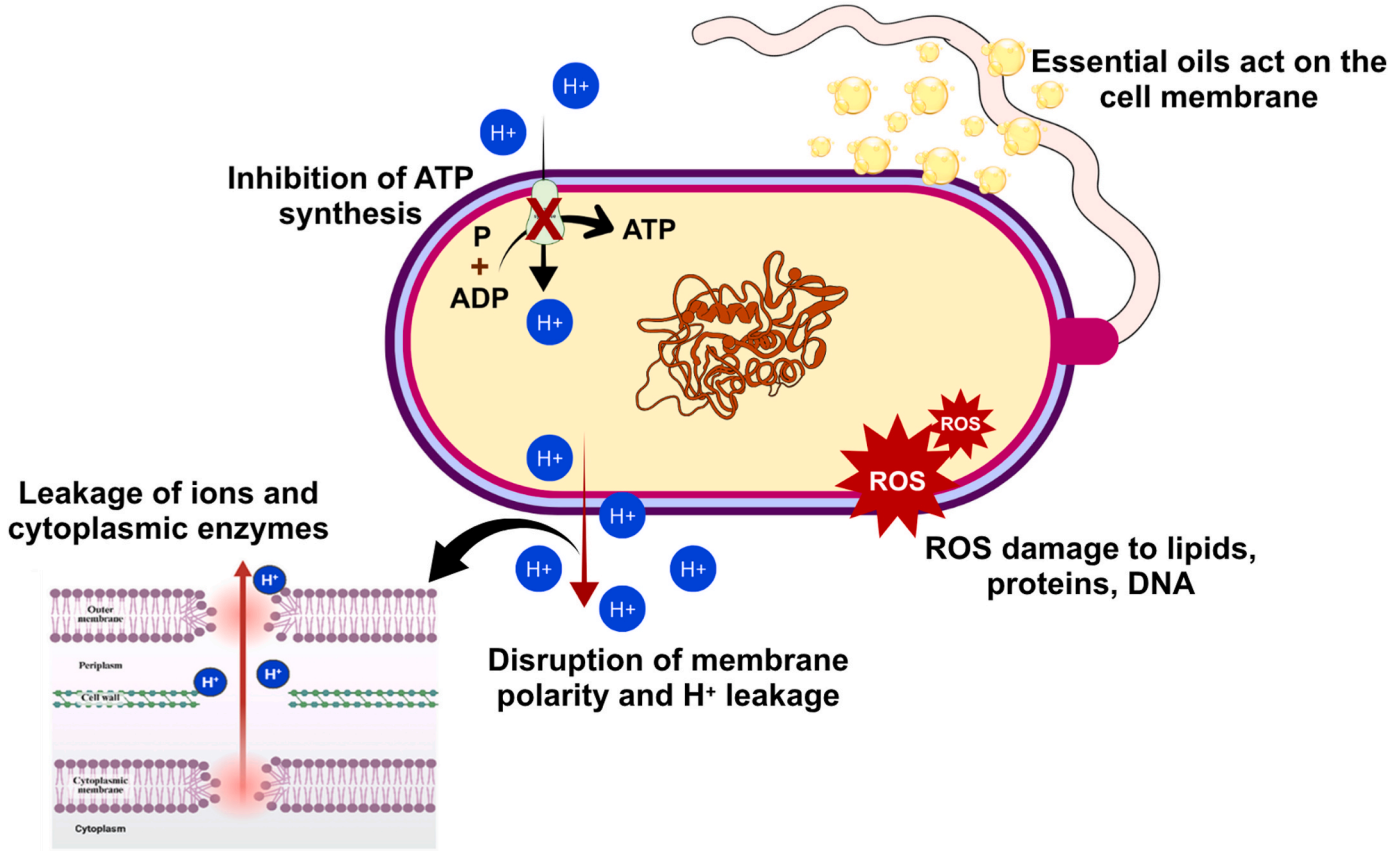


**Fig. 2.** Mechanism of action of natural antimicrobials against foodborne pathogens. Natural antimicrobials, particularly essential oils, interact with microbial membranes, altering polarity and dissipating the proton motive force (PMF) through hydrogen ($H^+$) ion leakage. This collapse of the electrochemical gradient inhibits adenosine triphosphate (ATP) synthesis and reduces cellular energy generation. Concurrently, essential oils induce the production of reactive oxygen species (ROS), which damage lipids, proteins, and deoxyribonucleic acid (DNA). In combination with leakage of cytoplasmic enzymes and ions, these effects compromise membrane integrity and metabolic functions, ultimately leading to microbial cell death. Symbols ( ) mean inhibition.

et al., 2021).

### 5.1. Fruits and vegetables

Edible films and coatings loaded with natural antimicrobials act as effective barriers that reduce water evaporation, slow respiration rates, and consequently extend the freshness of fruits and vegetables. Polysaccharide-based coatings combined with essential oils from herbs such as thyme, oregano, and clove have demonstrated efficacy in preventing spoilage and enzymatic browning (Ma et al., 2024). Moreover, plant extracts rich in polyphenols and flavonoids are incorporated into edible films to enhance their functional properties. These bioactive films have shown potent antimicrobial activity against *L. monocytogenes*, *E. coli*, and various *Penicillium* species (Guimarães et al., 2018). For instance, coatings derived from asparagus waste extract significantly extended the shelf life of strawberries, reduced weight loss, and prevented infections by fungi (Gupta et al., 2024). Similarly, cellulose nanofibers combined with antimicrobial carbon dots effectively suppressed fungal growth on tangerines and strawberries.

Given their volatility and hydrophobicity, essential oils are often embedded in polymer matrices to enhance their stability and antimicrobial efficacy in packaging applications. For example, cumin essential oil incorporated into edible starch films improved mechanical strength and antioxidant enzyme activity in coated fruits, delaying spoilage during storage. Furthermore, silver nanoparticles synthesized using bacteriocins from probiotic bacteria have been successfully applied in tomato packaging, exhibiting strong antimicrobial effects (Karnwal & Malik, 2024).

Building on these material-level strategies, several case studies have demonstrated their effectiveness in real food matrices. For example, Thyme essential oil nanoemulsion at 0.1 % eliminated fungal contamination of strawberries by 3 log CFU/g after 14 days at 4 °C, without affecting firmness or consumer acceptance (Kebriti et al., 2025). Similarly, a 1 % mint essential oil edible coating applied to cucumbers reduced *E. coli* and *Salmonella* by 2.5 log CFU/g within 7 days at 4 °C, with no adverse sensory impact (Morachis-Valdez et al., 2021). In lettuce, 0.2 % lemongrass essential oil vapor-phase treatment decreased *L. monocytogenes* by 2 log CFU/g over 10 days at 4 °C, while sensory analysis confirmed the absence of off-flavors, supporting its suitability for fresh-cut produce (Noshirvani, 2024).

### 5.2. Dairy products

Bacteriocins and lysozyme are key natural antimicrobials used to inhibit spoilage and pathogenic microorganisms in dairy products, thereby improving safety and shelf life (Rabbani et al., 2025). Lactic acid bacteria (LAB) produce bacteriocins antimicrobial peptides active against pathogens such as *L. monocytogenes*, *S. aureus*, and *Clostridium* species, which can be applied as purified compounds or integrated into bioactive films and coatings (Khaliq & Rabbani, 2025; Pujato et al., 2024). Control of pH and temperature further optimizes their antimicrobial efficacy in milk, yogurt and cheese (Silva et al., 2018).

Lysozyme, abundant in mammalian milk, hydrolyses the β-glycosidic linkages of the peptidoglycan layer of Gram-positive bacteria, disrupting their cell walls and preventing proliferation in cheese and pasteurized milk (Khaliq et al., 2024). Modified forms such as lysozyme-dextran

complexes exhibit improved solubility and surfactant properties, enhancing activity against Gram-negative bacteria. Synergistic combinations of lysozyme with lactoperoxidase or nisin further amplify antimicrobial effects, reducing microbial loads in dairy products (Khorshidian et al., 2022).

In addition to protein- and enzyme-based antimicrobials, plant-derived compounds such as essential oils have also been tested in dairy matrices. In dairy desserts, nanoemulsions of essential oils (1 %–15 %) markedly decreased microbial and yeast/mold counts, with higher doses yielding stronger microbial control and improved consumer acceptance compared to both controls and free oils (Nanakali, 2023). In curd cheese, Thymbra spicata essential oil (EO) nanoemulsions (1 %–5 %) inhibited yeast and mold growth for 28 days, significantly reducing spoilage microbes while maintaining favorable flavor and liking scores (Kayiran et al., 2025).

### 5.3. Meat and poultry

Organic acids, essential oils and chitosan are commonly applied to meat and poultry to inhibit bacterial contamination and extend shelf life. Organic acids such as lactic, acetic, and citric acids exert antimicrobial activity by lowering pH and penetrating microbial cell membranes, dissociating intracellularly to acidify the cytoplasm and inhibit enzymatic functions (Sorathiya et al., 2025). Treatment with organic acids effectively controls spoilage and pathogenic bacteria including *Pseudomonas*, Enterobacteriaceae, and aerobic mesophilic bacteria in refrigerated beef, pork, and poultry (Ben Braïek & Smaoui, 2021).

Essential oils extracted from thyme, oregano, clove, and *Artemisia* species possess antioxidant and antimicrobial properties, disrupting microbial membranes, inducing leakage of cellular contents, and generating reactive oxygen species. Nanoencapsulation techniques enhance their stability and prolonged efficacy. For instance, *Artemisia fragrans* essential oil incorporated into chitosan films reduced microbial growth and lipid oxidation in refrigerated chicken fillets, extending shelf life while maintaining sensory quality (Lotfy et al., 2023).

Chitosan derived from seafood waste exhibits antimicrobial properties by interacting with microbial cell surfaces, thereby increasing membrane permeability and leading to the leakage of vital intracellular contents. When applied as films on meat products, chitosan creates a barrier that limits oxygen transmission and inhibits microbial infiltration. Its application in combination with natural additives such as grape seed extract (GSE) or kombucha has demonstrated enhanced preservation efficacy. For instance, chitosan-GSE composite films effectively inhibit *L. monocytogenes*, *E. coli*, *S. aureus*, and molds on salmon and minced beef (Mojaddar Langroodi et al., 2021).

Nanoemulsions of cinnamon (89 nm) and curcuma (151 nm) essential oils significantly inhibited bacterial growth and lipid oxidation in minced beef, resulting in improved color stability and sensory quality compared with untreated samples (Jeong et al., 2021; Dghais et al., 2023). Similarly, in ostrich meat, a chitosan-based coating containing 2.5 %–5 % nanoemulsified *Eryngium campestre* essential oil effectively suppressed microbial growth during 12 days of refrigerated storage, while enhancing tenderness, juiciness, and overall sensory acceptance (Venkatachalam et al., 2025).

### 5.4. Seafood products

Natural antimicrobials such as seaweed extracts, chitosan, and organic acids have been extensively studied for seafood preservation due to their antibacterial and antioxidant properties. Brown and red seaweeds, including *Ascophyllum nodosum* and *Palmaria palmata*, contain bioactive compounds such as polysaccharides, polyphenols, phlorotannins, fatty acids, and pigments that inhibit seafood pathogens like *P. aeruginosa*, *S. aureus*, *E. coli*, and *Vibrio* spp. (Hejna et al., 2024; Teixeira et al., 2024). The antimicrobial efficacy of seaweed-derived polysaccharides depends on their molecular weight, sulfate content, and structural composition, while seasonal and geographical variations influence bioactive concentrations, affecting antimicrobial potency (Hejna et al., 2024).

Chitosan is effective against several microorganisms due to the positive charges on its sugar chains. These charges interact electrostatically with microbial membranes, increasing permeability and disrupting DNA synthesis (Mei et al., 2020). Coatings and films containing chitosan have reduced microbial loads including coliforms, *Aeromonas*, *Pseudomonas*, and *Vibrio* in refrigerated fish fillets, thereby extending shelf life (Previdi et al., 2018). Organic acids such as lactic and acetic acids are also applied to acidify the product surface, disrupt microbial cells and delay spoilage. Their combined use with seaweed extracts or chitosan enhances preservation efficacy while maintaining desirable sensory attributes.

In addition to polysaccharides and acids, essential oil nanoemulsions have shown great promise in seafood preservation. Curcumin and rosemary oil nanoemulsion extended the shelf life of fish fillets from 6 days (control) to 27 days at 4 °C, by reducing microbial spoilage, slowing pH increase, and preserving superior texture and flavor. Sensory panel evaluation confirmed significantly higher acceptability compared with untreated fish, highlighting the potential of essential oil nanoemulsions to extend seafood freshness without compromising consumer appeal (Ceylan et al., 2020).

### 5.5. Baked goods and cereals

Essential oils and phenolic compounds are increasingly employed in baked goods and cereal products for their antifungal and antimicrobial activities, protecting products from spoilage over extended storage periods. Essential oils, containing volatile plant constituents such as terpenes, phenols, and alcohols, are extracted from plants like oregano, thyme, clove, cinnamon, and rosemary. These compounds disrupt microbial membranes, causing leakage of intracellular contents and microbial death, while also providing antioxidant effects that prevent lipid oxidation in cereals and baked products (Gavahian et al., 2020). The incorporation of EOs and phenolics with polysaccharides such as chitosan, cellulose, or starch enhances film mechanical strength, reduces moisture and gas permeability, and imparts antimicrobial and antioxidant functionality. Polysaccharide-protein composites stabilizing Pickering emulsions represent a promising approach for essential oil-based sustainable packaging, effectively protecting baked goods and cereals from fungal contamination without imparting undesirable odors or flavors. For instance, the surface application of oregano and clove essential oils to bread inhibited fungal growth and extended shelf life (De Farias et al., 2025; Noshirvani, 2024).

Building on these findings, nanoemulsion-based systems have further improved the efficacy of essential oil applications in bakery products. Application of cinnamon and clove essential oil nanoemulsions to muffins prolonged shelf life by more than 12 days, inhibiting microbial growth and reducing moisture/oxygen transfer. These treatments also conferred higher antioxidant capacity while preserving desirable texture and flavor (Prastuty et al., 2022).

In addition to essential oils, phenolic compounds represent another major class of natural bioactives applied in baked and cereal products. Phenolic compounds, including flavonoids, tannins, and phenolic acids, naturally abundant in plants, exhibit potent antimicrobial and antioxidant activities by damaging fungal cells and inhibiting essential enzymatic functions necessary for growth and reproduction. Polyphenols extracted from grape seeds, green tea, and similar sources are utilized in cereal products and films to delay fungal contamination and extend shelf life. Current research focuses on preserving the bioactivity of phenolics and enhancing their application in food matrices (Molnar et al., 2023) The practical implementation of these compounds in various food categories is illustrated in Fig. 3.

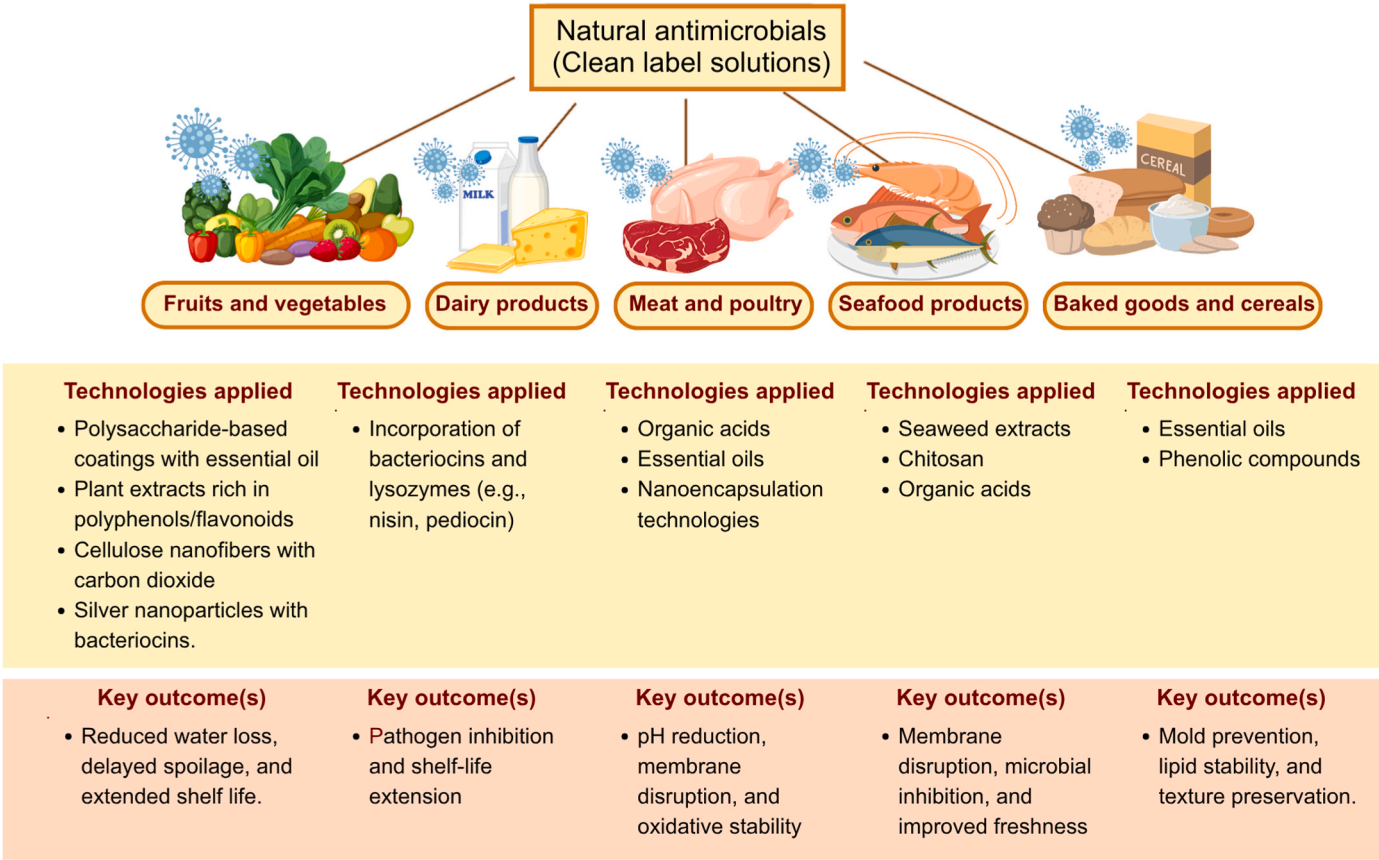


**Fig. 3.** Applications of natural antimicrobials across in major food categories. The figure illustrates the application of natural antimicrobials (clean-label solutions) in fruits and vegetables, dairy products, meat and poultry, seafood, and baked goods/cereals. Each category shows representative technologies and key outcomes such as shelf-life extension, pathogen inhibition, or quality preservation.

## 6. Factors influencing the efficacy of natural antimicrobials

### *6.1. Concentration, dosage, and antimicrobial efficacy*

The antimicrobial effectiveness of natural compounds is strongly influenced by their concentration and dosage. Many natural antimicrobials, including essential oils, bacteriocins, chitosan, and plant-derived extracts, exhibit dose-dependent behaviour, where higher concentrations often yield stronger inhibitory effects (Rabbani et al., 2024). However, excessive amounts may negatively impact the sensory properties or safety of food products. Minimum inhibitory concentration (MIC) represents the lowest concentration of an antimicrobial agent required to inhibit visible microbial growth and provides a useful benchmark. However, they are often lower than the concentrations needed in actual food systems. This discrepancy is largely due to the complex composition of foods, where interactions with lipids, proteins, and carbohydrates can hinder the bioavailability and efficacy of antimicrobials (Vipra et al., 2013). For example, although chitosan demonstrates broad-spectrum activity at 0.5 % (w/v) against both Gram-positive and Gram-negative bacteria in laboratory media, higher concentrations are typically required when applied in real food matrices. Similarly, nisin exhibits effective inhibition of *L. monocytogenes* at concentrations as low as 25 μg/mL in liquid media, but higher amounts are necessary in foods due to limited nisin diffusion (Karnwal & Malik, 2024).

The effective concentration of natural antimicrobials varies depending on the target microorganism and the specific food matrix. Bacteriocins such as nisin are particularly active against Gram-positive bacteria including *Bacillus* spp., *S. aureus*, and *L. monocytogenes*, typically at concentrations ranging from 10 to 50 μg/mL in meat and cheese products. However, in complex food systems, higher dosages may be required to maintain efficacy without compromising sensory attributes. In contrast, Gram-negative bacteria such as *E. coli*, *Salmonella* spp., and *Pseudomonas* spp. exhibit greater resistance due to the presence of an outer membrane barrier. Effective inhibition in seafood, fruits, and vegetables often requires the application of chitosan at 0.5 %–1 % (w/v) or essential oils at 0.1 %–1 % (v/v). Similarly, fungal contamination in baked products can be mitigated by incorporating thyme or clove essential oils at concentrations of 0.05 %–0.5 % (v/v); however, their strong aromas may alter the product's sensory profile. To overcome such limitations, techniques such as nanoencapsulation, synergistic blending of antimicrobial agents, and food-specific dosage optimization are employed to enhance antimicrobial efficacy while minimizing sensory impacts (Sar et al., 2023).

### *6.2. Food matrix composition*

The efficacy of natural antimicrobials is not only determined by their inherent potency, but also by the physicochemical characteristics of the food matrix into which they are incorporated. Critical factors such as fat content, pH, water activity (aw), and protein interactions can significantly modulate antimicrobial performance, either enhancing or impeding their activity in real food environments.

#### *6.2.1. Fat content and pH*

The antimicrobial efficacy of natural compounds is significantly influenced by the physicochemical properties of the food matrix, particularly fat content and pH. In high-fat dairy and meat products, higher concentrations of oregano or clove essential oils are required to effectively inhibit the growth of *L. monocytogenes* and *Salmonella* spp.

due to the solubilization of hydrophobic components by fat molecules, which reduces their availability at the microbial interface (Zheng et al., 2024). This sequestration effect limits the interaction of compounds such as carvacrol and thymol with microbial membranes in aqueous environments. In such matrices, fat globules can encapsulate bacterial cells, creating physical barriers that hinder antimicrobial access (Santiesteban-López et al., 2022).

The pH of the food also plays a critical role in antimicrobial performance. Acidic environments enhance the activity of organic acids and bacteriocins due to increased protonation and improved membrane permeability (Niroula et al., 2024). For instance, in dairy products with pH values below 5.5, nisin exhibits greater inhibitory activity against *C. botulinum* and *L. monocytogenes* than in neutral conditions. Similarly, lactic and acetic acids demonstrate higher efficacy at low pH, as their undissociated forms can penetrate bacterial membranes and disrupt intracellular processes. The antimicrobial action of lysozyme is also potentiated in acidic foods but attenuated near neutrality (Karnwal & Malik, 2024). In contrast, extremely acidic or alkaline conditions may cause degradation or structural alteration of some antimicrobials, diminishing their stability and activity. Therefore, understanding food matrix parameters such as fat content and pH is essential for tailoring the effective use of natural antimicrobials in food systems.

#### 6.2.2. Water activity and protein interactions

The interaction dynamics between microorganisms and natural antimicrobials are substantially affected by the water activity (aw) of the surrounding environment. While low aw effectively suppresses bacterial proliferation, it may also hinder the release and diffusion of antimicrobial agents, thereby compromising their efficacy. In low-moisture foods such as dried cereals and cured meats, limited water availability delays or reduces the interaction between antimicrobial compounds and microbial cells. For instance, chitosan remains effective across a broad range of aw values, owing to its ability to form a semi-permeable film that impedes microbial adhesion and growth (Zhao et al., 2020).

Additionally, the antimicrobial bioactivity of natural compounds can be reduced through binding with proteins within the food matrix. Proteins present in milk and meat, for example, may bind to phenolic compounds and essential oils, thereby limiting their availability and impeding their interaction with microbial membranes. Consequently, studies have indicated that protein-rich foods such as cheese and meat often require higher concentrations or more potent antimicrobials to overcome the binding effects and maintain microbial control (Shahidi & Dissanayaka, 2023).

### 6.3. Synergistic and antagonistic interactions

Natural antimicrobials are increasingly employed in food preservation owing to their safety and efficacy. However, their performance can be significantly influenced by interactions with other food components, additives, and preservation technologies. Depending on the nature of these interactions, antimicrobial agents may exhibit synergistic effects, enhancing each other's efficacy, or antagonistic effects, reducing their individual performance. Understanding these interactions is crucial for designing reliable and effective preservation strategies.

#### 6.3.1. Synergistic interactions

Synergy occurs when the combined efficacy of two or more antimicrobials exceeds the sum of their individual effects. Such combinations not only broaden the antimicrobial spectrum but also reduce the quantity required for each agent. Consequently, this approach reduces production costs and minimizes undesirable sensory changes in food products. Combining natural antimicrobials with synthetic preservatives has demonstrated significant improvements in antimicrobial effectiveness. For instance, cinnamon essential oil combined with sodium benzoate or potassium sorbate significantly enhanced inhibition of *L. monocytogenes* and *E. coli* in fruit juices and meat products compared to individual applications (Quinto et al., 2019).

Essential oil blends have similarly demonstrated improved antimicrobial efficacy. Mixtures of thyme, oregano, rosemary, and sage essential oils have effectively inhibited *Salmonella* spp. and *L. monocytogenes*. Notably, combining thyme and oregano oils with modified atmosphere packaging (MAP) significantly reduced mesophilic bacterial populations on sprouts and cabbage, exceeding the efficacy of essential oils alone. Such synergy allows lower essential oil concentrations, thereby minimizing strong aromas and preserving desirable sensory properties of the food (Karnwal & Malik, 2024). Another synergistic strategy involves synergistic pairings of protein-based antimicrobials, such as nisin and pediocin, with essential oils. These combinations have shown enhanced efficacy against drug-resistant bacteria. For instance, combining Allium-derived antimicrobial proteins with clove or cinnamon oils broadens the inhibition spectrum, effectively targeting *S. aureus* and *E. coli*. Such approaches are especially useful in complex food matrices where traditional agents may fail (Soltani et al., 2022). Furthermore, incorporating natural antimicrobials into films or coatings for food packaging also ensures sustained efficacy over extended storage periods. Chitosan films containing plant extracts or essential oils significantly inhibit *Pseudomonas* and *Aeromonas* in refrigerated fish products. Such encapsulation protects antimicrobial agents from environmental degradation and facilitates their controlled release, thus significantly enhancing microbial control (Quinto et al., 2019).

#### 6.3.2. Antagonistic interactions

Conversely, not all combinations of antimicrobials result in enhanced effectiveness. Certain combinations may exhibit antagonistic interactions, wherein one compound diminishes or counteracts the activity of another. A common antagonistic mechanism involves the binding of antimicrobial agents to components within the food matrix, such as proteins, lipids, and carbohydrates. For instance, phenolic compounds and essential oils can bind to milk caseins or meat myofibrillar proteins, thereby reducing their antimicrobial effectiveness (Quinto et al., 2019). As a result, higher antimicrobial concentrations may be required, which could adversely affect the organoleptic properties of food products. Table 3, presents selected combinations of natural antimicrobials and additives, their food applications, microbial targets, interaction types, and effects on bacterial cells. In addition to food matrix interactions, chemical degradation is another factor that compromises antimicrobial efficacy. Many phenolic compounds are sensitive to environmental factors such as light, oxygen, and extreme pH, which may degrade their chemical structure and diminish both antimicrobial and antioxidant activities. Furthermore, certain food additives or preservatives may interact unfavorably with natural antimicrobials, leading to reduced stability and overall effectiveness (Pasquet et al., 2024).

## 7. Application strategies for natural antimicrobials

### 7.1. Surface treatment

Surface application involves the delivery of natural antimicrobials onto food surfaces via dipping, spraying, or coating. The antimicrobial efficacy primarily relies on direct contact with microorganisms, facilitating membrane disruption and metabolic interference. Essential oils and chitosan, for instance, interact with microbial membrane lipids, increasing permeability and causing leakage of ions, proteins, and nucleic acids, ultimately leading to cell death (Nasaj et al., 2024).

Edible coatings composed of biopolymers such as chitosan or alginate, often enriched with natural agents like thyme and oregano, have shown efficacy in reducing spoilage organisms including *Pseudomonas*, *L. monocytogenes*, and *Salmonella* spp. on fruits, vegetables, seafood, and meat. These coatings are typically applied via dipping or spraying. For example, fish fillets treated with chitosan–herbal extract solutions exhibited reduced *Aeromonas* and *Vibrio* counts during chilled storage

**Table 3**
Overview of combined preservative effects on bacterial cells: synergy and antagonism.

| Combination | Food product | Microorganisms affected | Interaction | Effect on bacterial cell | References |
|---|---|---|---|---|---|
| Cinnamon EO + Sodium benzoate | Fruit juices, meat | *Listeria monocytogenes*, *E. coli* | Synergistic | Enhanced Inhibition of microbial growth | Quinto et al. (2019) |
| Thyme + Oregano + MAP gases | Sprouts, cabbage | Mesophilic bacteria | Synergistic | Complete inhibition of bacterial growth | Karnwal and Malik (2024) |
| Nisin + Essential oils | Dairy products | *Staphylococcus aureus*, *Salmonella* | Synergistic | Decrease in microbial growth | (Moosavy et al., 2008) |
| Chitosan + Plant extracts | Fish fillets | *Pseudomonas*, *Aeromonas* | Synergistic | Increased shelf life | (Rathod et al., 2021) |
| Phenolics + Milk products | Cheese, dairy products | Different types of spoilage organisms | Antagonistic | Reduced microbial growth | Ullah et al. (2022) |
| High EO concentrations | Bakery products | Molds and yeasts | Antagonistic | Off- flavours limiting consumer acceptance | Pasquet et al. (2024) |

(Deshmukh & Gaikwad, 2022). Similarly, the application of nisin and plant-based antimicrobials on fresh-cut fruits effectively delays the growth of molds and pathogenic bacteria (Cheruvari & Kammara, 2025; Khaliq et al., 2023). The primary advantage of this method lies in its direct targeting of surface-residing microorganisms, with the dosage and composition easily tailored to specific product needs. However, the efficacy may diminish over time due to interactions with food surfaces or environmental factors (Guimarães et al., 2018).

### 7.2. Direct incorporation into food matrices

In direct incorporation, natural antimicrobials are mixed into the food matrix during processing, enabling homogeneous distribution and internal protection against spoilage and pathogenic microorganisms. The mechanism of action varies depending on the antimicrobial used. Organic acids such as lactic and citric acid penetrate microbial membranes, acidify the cytoplasm, and disrupt enzymatic functions (Lucera et al., 2012). Bacteriocins like nisin interact with lipid II on the bacterial membrane, forming pores that cause ion leakage and ATP depletion (Sharma et al., 2021). This method is commonly employed in dairy, meat, and beverage production to control pathogens such as *L. monocytogenes* and *Clostridium* spp. (Mahmud & Khan, 2018). In bakery and cereal products, essential oils and plant extracts are often incorporated to inhibit mold growth and prolong freshness. However, interactions with proteins and lipids may reduce antimicrobial efficacy, and high concentrations may alter sensory properties. Despite these limitations, direct incorporation remains a practical and efficient method for enhancing food safety and shelf life (Lucera et al., 2012).

### 7.3. Integration into packaging systems

Embedding natural antimicrobials into packaging materials is a widely adopted strategy for developing active packaging systems. These materials release antimicrobial compounds gradually onto the food surface, inhibiting microbial growth and delaying spoilage (Deshmukh & Gaikwad, 2022). Most antimicrobial agents exert their effects through membrane disruption, interference with energy metabolism, and induction of oxidative stress. Chitosan-based films enriched with essential oils or plant extracts have demonstrated prolonged antimicrobial activity in fish fillets, maintaining product safety during storage.

Encapsulation of antimicrobials within polymer matrices protects them from premature degradation and facilitates sustained release. This approach maintains the sensory and quality while reducing the need for synthetic preservatives (Duda-Chodak et al., 2023). However, challenges remain regarding production scalability, regulatory approval, and precise control of release kinetics to avoid overdosing (Brandelli, 2024). Table 4 demonstrate a solution framework linking instability modes of natural antimicrobials to suitable delivery systems. Safety and Toxicology. While many natural antimicrobials are GRAS, their toxicological profiles require systematic evaluation. Acceptable Daily Intakes (ADIs) are available for some compounds (e.g., sorbates, benzoates) but remain unclear for most essential oils and phenolics. *In vivo* studies show that bacteriocins such as nisin are well tolerated, whereas spice-derived oils may cause allergenicity or irritation at high doses. Chitosan is safe in most cases, but its crustacean origin poses potential allergen risks. Sublethal exposure may induce stress responses, promote biofilm persistence, and contribute to antimicrobial resistance. Reports indicate that low concentrations of essential oils can enhance tolerance in pathogens such as *L. monocytogenes* and *Salmonella (de Medeiros Barbosa et al., 2016)*.

Hurdle technology represents a rational strategy to enhance the efficacy of natural antimicrobials while minimizing sensory and toxicological drawbacks. Rather than considering hurdles as isolated interventions, their combinations should be systematically designed to achieve targeted microbial control within specific food matrices (Yu et al., 2023). For instance, essential oils combined with modified atmosphere packaging (MAP) have demonstrated synergistic inhibition of *L. monocytogenes* and *E. coli* O157:H7, while maintaining texture and reducing oxidative changes (Xiong et al., 2020). Similarly, bacteriocins such as nisin and pediocin, when applied together with mild high-pressure processing (HPP; <400 MPa), effectively suppress *C. botulinum* spores and *L. monocytogenes* with minimal sensory changes (Modugno et al., 2019). Chitosan-based edible films enriched with organic acids provide controlled release against *P. fluorescens and Shewanella putrefaciens*, therby extending shelf life without causing excessive acidification (Chandarana et al., 2025). Moreover, plant-derived phenolics on combination with lactate salts enhance inhibition of *S. enterica* and *L. monocytogenes*, while reducing the strong off-flavors often associated with essential oils (Oulahal & Degraeve, 2022).

### 7.4. Vapor-phase delivery

Vapor-phase application involves dispersing volatile natural antimicrobials, primarily essential oils, into the headspace of packaging environments. The vapours interact with surface-residing microbes without direct contact with the food product. This non-contact delivery method has been effective in extending the shelf life of bakery items and fresh produce (Laird & Phillips, 2012). Essential oil vapours such as cinnamon, clove, and thyme have shown strong antifungal and antibacterial properties, particularly when combined with reduced-oxygen or modified-atmosphere packaging. This approach maintains the food's physical integrity and sensory attributes by avoiding direct chemical interaction. However, challenges include the volatility and potential sensory impact of essential oils at high concentrations, which may impart off-flavours or odors. Careful formulation and vapor release control are essential for commercial viability (Gavahian et al., 2020). The practical application strategies of natural antimicrobials in food preservation are summarized in Fig. 4.

**Table 4**
A solution framework linking instability modes of natural antimicrobials to suitable delivery systems.

| Instability mode | Why it matters | Delivery solutions | Application in food additives | References |
|---|---|---|---|---|
| Volatility | Substance might evaporate during processing, loss of antimicrobial activity | Nanoemulsion/nanocapsules: droplet size 50–200 nm; encapsulation efficiency ≥70 %; oil:surfactant (w/w) 1:1 → 1:3; high-pressure homogenization 100–200 MPa (or ultrasonication 5–15 min, 40 % amplitude) to produce stable O/W nanoemulsions. Vapor-phase with controlled-release sachets for headspace dosing when direct contact is undesirable. | Thyme, oregano, clove | Chen et al. (2020) |
| Oxidation | Antimicrobial activity might get lost and offensive odour might form | Co-encapsulation + antioxidant matrix: encapsulate active in polymer/lipid core (e.g., polymeric nanoparticle or liposome) containing co-antioxidant (e.g., tocopherol, ascorbyl palmitate). Use oxygen-barrier films or include oxygen scavenger sachet in package. Aim for particle sizes <200 nm to reduce diffusion and increase retention. | Carvacrol, eugenol in films for meat/fish, phenolics in bakery | Chawda et al. (2017) |
| Heat/pH sensitivity | Activity lost due to high processing temperature, non-optimal pH can cause denaturation of food | pH-/thermo-responsive microcapsules & liposomes: protein/polysaccharide matrices (e.g., whey protein, alginate) are used to create microcapsules, which keep them stable until the target conditions are met; microcapsule diameter 1–1000 00 -1crosslinked shell liposomes or polymeric NPs (100–200 nm) are a crosslinked shell used in nanosystems. The actives are preserved by drying (spray-dry) at less than 80 C inlet. | Nisin/pediocin in cheese/meat, lysozyme in dairy coatings | Das et al. (2021) |
| Photo degradation | UV light can cause rapid breakdown of food particles | Opaque or UV-blocking active film/encapsulation: add UV absorbers to polymer, apply multilayer films or add actives to halloysite or other inorganic nanotubes; store finished product in an opaque/UV-filtered package. | Light-sensitive phenolics in clear packaging for juices | Ullah et al. (2022) |
| Matrix Sequestration | Active partitions into fat/protein; unavailable at microbial interference | Carrier enhancing dispersion & interfacial activity: nanoemulsions, cyclodextrin inclusion complexes, halloysite nanotubes or surfactant-stabilized nanocarriers to decrease particle size to less than 200 nm diameter to maximize contact area | Essential oil in high-fat meat; nanoemulsion coatings | Quinto et al. (2019) |
| Sensory Impact | If the dose is not proper, it leads to consumer rejection | Controlled/sustained release (nano/microencapsulation in edible coating); multiple layer films, which release gradually; inclusion in synergists to reduce dose required; cyclodextrin -complexation of aroma. Common coating composition: chitosan 0.5–1.5 (w/v), alginate 1–2 (w/v), plasticizer (glycerol) 0.5–1.5 (w/w); use by dipping/spraying (immersion 30–60 s; air-dry). | Oregano/thymol for fruits and bakery | Xing et al., 2016 |

## 8. Challenges and limitations associated with natural antimicrobials

### 8.1. Impact on sensory properties

Natural antimicrobials, such as essential oils and phenolic compounds, have become popular in food preservation due to their broad antimicrobial and antioxidant activities. However, their application is sometimes limited by adverse impacts on the sensory characteristics of food, including taste, color, and texture. Although essential oils effectively inhibit bacteria, fungi, and mycotoxins, their high volatility and strong aroma profiles significantly altered natural taste of foods. For instance, cinnamon, clove, oregano, and thyme oils effectively control pathogens in dairy products and fruit juices but often impart intense flavors that may negatively affect consumer acceptance (Gavahian et al., 2020). Phenolic compounds, abundant in spices and plant extracts, possess antioxidative properties but may also initiate enzymatic browning and produce undesirable aromas (Derardja et al., 2022). In fruits, vegetables, and meats, polyphenoloxidase-mediated oxidation of phenolics can lead to dark pigmentation and off-flavors, reducing visual appeal and consumer acceptability. Ferulic and sinapic acids, two common phenolic acids, can degrade into 4-vinyl guaiacol, contributing off-flavors in stored citrus products. Additionally, improperly processed seed meals rich in phenolics can impart astringency and dark coloration to meat products (Konwar et al., 2024).

### 8.2. Regulatory and safety concerns

The use of natural antimicrobials derived from plants, microorganisms, animals, and marine sources is gaining increasing attention due to their perceived safety and environmental sustainability compared to synthetic chemical preservatives. While many natural antimicrobials have been granted GRAS status, significant regional variation in food safety regulations has led to inconsistencies in the approval of specific compounds, permissible concentrations, and allowable food applications. This regulatory complexity poses substantial challenges for food manufacturers in standardizing formulations and obtaining multi-regional market approvals (Öztekin et al., 2023). Table 5 summarizes the regulatory status and application limits of natural antimicrobials across regions, highlighting differences in GRAS designation, EU approval, permissible concentrations, and labeling requirements.

Excessive or prolonged use of certain natural antimicrobials may raise safety concerns, including allergic reactions, toxicological effects, or disruptions to the gut microbiota. Appropriate processing measures must be implemented to eliminate naturally occurring toxins from these sources (Zhang et al., 2019). Furthermore, the incorporation of natural antimicrobials can alter the flavor, aroma, and visual appearance of food products. If these sensory attributes are not properly controlled, they may lead to reduced consumer acceptance (Aziz & Karboune, 2018). To address these challenges, further research is needed to develop improved methods for isolating and purifying natural antimicrobials, investigate their safety profiles, and establish standardized regulatory frameworks for their safe and effective use (Pessoa et al., 2021).

Beyond regulatory and toxicological considerations, practical application also requires careful formulation design, as not all hurdle combinations yield positive interactions. Polyphenolic compounds may chelate ions, thereby reducing bacteriocin activity. Similarly, excessive concentrations of essential oils in MAP systems can impart overpowering aromas, while high organic acid levels in films may cause surface whitening or adverse textural changes. These examples highlight the need for balanced formulation, matrix-specific optimization, and sensory validation when designing hurdle-based systems (Todorov et al., 2025). Effective hurdle design requires: (i) selecting antimicrobials based on pathogen physiology (e.g., bacteriocins for Gram-positive bacteria, organic acids for Gram-negative bacteria), (ii) applying each factor at sub-inhibitory levels to achieve synergy while minimizing

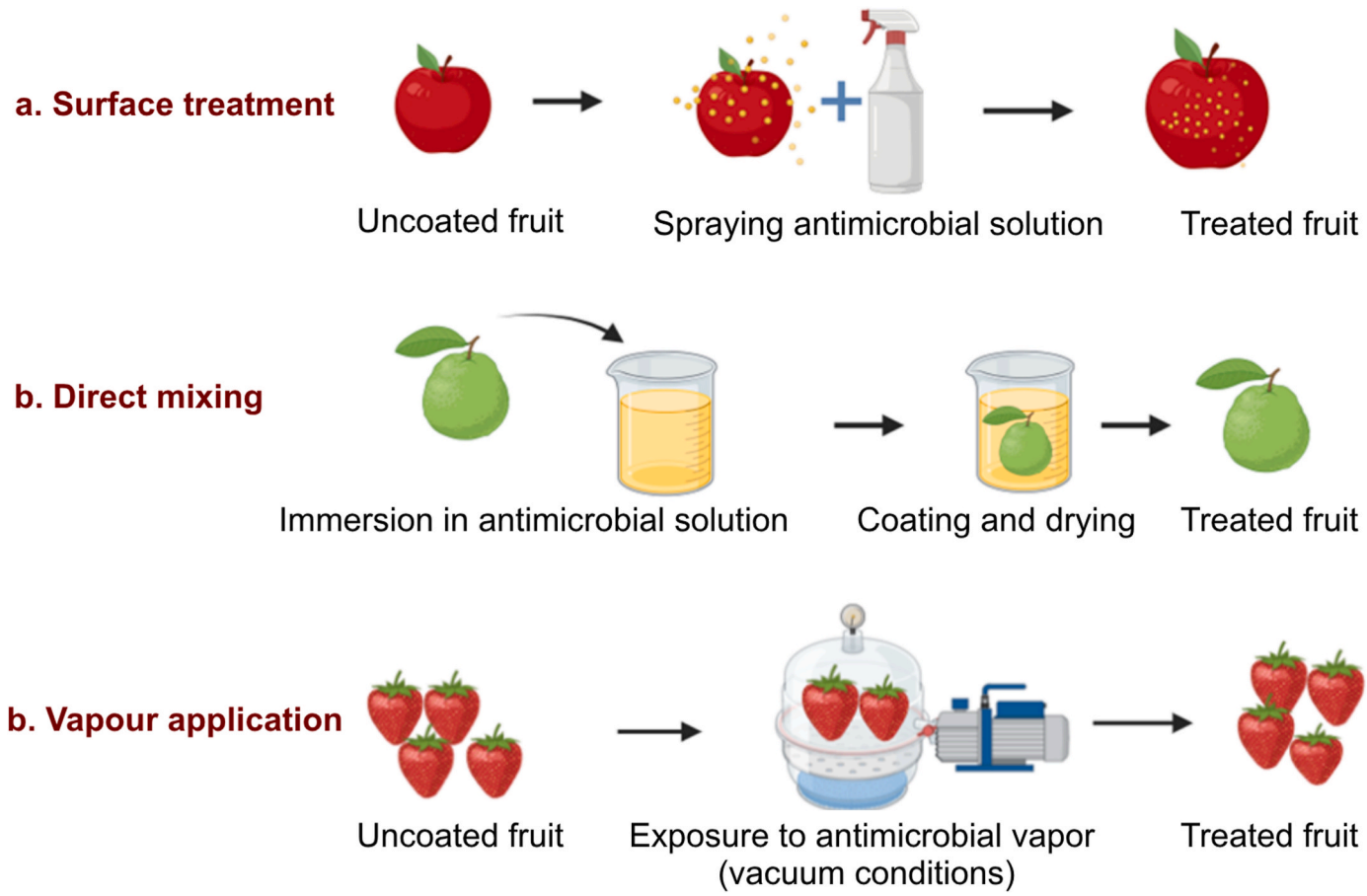


**Fig. 4.** Practical application strategies of natural antimicrobials to fruits. This figure illustrates three major approaches for applying natural antimicrobials to fresh produce: (a) Surface treatment: Spraying antimicrobial solutions onto fruit surfaces (e.g., apples) to create a protective barrier. (b) Direct mixing: Immersing fruits in antimicrobial solutions followed by coating and drying, ensuring uniform surface coverage (c) Vapor application: Exposing fruits (e.g., strawberries). to antimicrobial vapours under vacuum conditions, allowing uniform treatment without direct liquid contact. These application strategies enhance microbial inhibition, maintain fruit quality, and extend shelf life.

negative impacts, (iii) ensuring physicochemical compatibility with the food matrix, and (iv) incorporating sensory validation into efficacy testing (Choi et al., 2023).

### 8.3. Stability and shelf life

Although natural antimicrobials have potential over synthetic preservatives, their stability and sustained antimicrobial efficacy remain significant challenges. Many bioactive compounds are prone to rapid degradation upon exposure to environmental factors such as light, heat, oxygen, and pH fluctuations. Owing to their volatile nature, essential oils frequently lose potency during food processing and storage, thereby compromising their ability to control microbial growth. Phenolic compounds are similarly susceptible; extended exposure to oxygen and light can alter their chemical structure, adversely affecting both antimicrobial activity and sensory attributes (Soto-Vaca et al., 2012). To address these issues, innovative delivery systems such as nanoencapsulation, microemulsions, and edible coatings have been developed. These technologies help shield natural antimicrobials from environmental degradation, thereby maintaining their bioactivity and extending their shelf life. In addition to enhancing stability, these systems improve compatibility with complex food matrices and enable the controlled, sustained release of active compounds.

### 8.4. Cost and scalability

Despite the numerous benefits of natural antimicrobials, their large-scale commercial application remains limited due to economic constraints and scalability challenges. The extraction of natural bioactive compounds often involves costly, time-consuming, and technically complex processes, hindering their industrial-scale production (Krakowska-Sieprawska et al., 2022). Seasonal and geographical variability in raw material availability further complicates standardization, affecting both supply consistency and product efficacy. Due to their high production costs, natural antimicrobials are less feasible for use in cost-sensitive markets (Karnwal & Malik, 2024). To improve production feasibility, advancements in extraction techniques, yield optimization, and formulation strategies that preserve antimicrobial activity are essential. The integration of modern preservation technologies and advanced packaging solutions enables reduced antimicrobial dosage while maintaining the shelf life and safety of food products. Close collaboration among academia, industry stakeholders, and regulatory authorities is essential to address these challenges and support the commercial viability of natural antimicrobials (Yarmolinsky et al., 2024).

## 9. Recent advances in delivery system for natural antimicrobial

In recent years, considerable progress has been made in developing nano- and micro-encapsulation technologies to improve the stability, bioavailability, and controlled release of natural antimicrobials such as essential oils. These advanced delivery systems address critical limitations associated with the direct application of natural antimicrobials in foods, including volatility, sensitivity to environmental factors (e.g.,

**Table 5**
Regulatory status and application limits of natural antimicrobials across regions.

| Natural Antimicrobial | GRAS Status (U.S) | EU Approval Status | Use-Level Limits and Labelling notes | References |
|---|---|---|---|---|
| Thymol, Carvacrol | Generally Recognized As Safe (GRAS) when used as food additives and flavourings | Approved as flavourings and food additives within EU Regulation (EC)No 1334/2008 | Use concentration ranging from ≤0.05 % to 0.5 % v/v to avoid sensory impact | Karnwal & Malik, 2024 |
| Eugenol (Clove oil) | GRAS as a flavouring agent | Authorized flavouring under EU flavour regulations | Concentrations limited by sensory thresholds, potential allergen declaration under EU regulation (EU No 1169/2011) | Silva et al. (2024) |
| Lysozyme | GRAS status for use in cheese and dairy | Approved as a food additive | 400 μg/mL in cheese products due to allergenicity | Khorshidian et al. (2022) |
| Nisin | Not considered as GRAS officially, but found to be safe in certain fermented foods | Authorized in some EU member states but no full EU-wide approval | Maximum usage level vary by product type (i.e: upto 12.5 mg/kg in cheese) | Silva et al. (2018) |
| Chitosam | No current GRAS status; considered a dietary fibre and generally safe | Approved as a food additive under certain conditions (EU No 1129/2011) | Usage levels limited (typically <1 % w/v in coatings) | Chandarana et al. (2025) |
| Organic acids (Lactic acid, Acetic acid) | GRAS for use as food additives | Approved food additives (E270 for lactic acid, E260 for acetic acid) | Maximum use levels vary widely based on food type | Sorathiya et al. (2025) |

heat, oxygen, light), and interactions with food matrix components.

### 9.1. Nanoencapsulation

Nanoencapsulation involves the entrapment of bioactive antimicrobial compounds, such as essential oils, within nanocapsules or nanoemulsions typically ranging from 10 to 100 nm in size, enhancing their physical stability and antimicrobial efficacy (Rabbani, Srikumar, et al., 2025). Due to their ultra-small dimensions and high surface-area-to-volume ratio, nanoparticles exhibit superior interaction with microbial cells, leading to enhanced antimicrobial performance. This technology enables the controlled release of active compounds, allowing for sustained antimicrobial action while minimizing adverse impacts on the sensory properties of food products (Lelis et al., 2021). Encapsulated essential oils have been shown to disrupt microbial membranes, causing leakage of intracellular contents and subsequent cell death. Importantly, the encapsulation matrix prevents premature volatilization and degradation of the oils, thereby extending their antimicrobial functionality. For instance, nanoencapsulated essential oils have demonstrated prolonged inhibitory effects against *L. monocytogenes*, *E. coli*, and *Botrytis cinerea* in various food matrices. Additionally, nanoencapsulation shields antimicrobials from potential interactions with macromolecules such as proteins, lipids, and carbohydrates, which could otherwise reduce their bioavailability and effectiveness (Das et al., 2021). Moreover, the improved solubility of nanoencapsulated hydrophobic compounds in aqueous food systems contributes to their uniform dispersion and increased bioefficacy. Applications such as nano emulsion-based coatings incorporating thyme or clove essential oils have been successfully employed on postharvest fruits, effectively inhibiting fungal growth and mycotoxin production while preserving fruit firmness, color, and consumer appeal (Das et al., 2021). As summarized in Fig. 5, the fabrication of nanoencapsulated plant bioactives illustrates how this approach enhances stability, controlled release, and antimicrobial delivery efficiency.

### 9.2. Microencapsulation

Microencapsulation refers to the entrapment of natural antimicrobial agents within microparticles typically ranging from 1 to 1000 μm in diameter, using wall materials composed of proteins, polysaccharides (e.g., starch, cellulose), or lipids. This encapsulation approach effectively protects sensitive bioactive compounds from external stressors, while also reducing undesirable impact on the flavor or aroma of the final product (Niroula et al., 2024).

Microcapsules can be engineered to release their contents under specific environmental conditions, such as changes in pH, temperature, or enzymatic activity thereby allowing for controlled and targeted antimicrobial delivery. Studies have demonstrated that essential oils released from microencapsulated systems embedded in edible films or coatings can effectively inhibit microbial proliferation in meat and dairy products by compromising microbial cell wall integrity and interfering with ATP production (Mukurumbira et al., 2022). This technique offers the advantage of improved protection against premature degradation and enhances the dispersion of hydrophobic antimicrobials within aqueous food matrices. Consequently, effective microbial control can often be achieved at lower concentrations, minimizing sensory alterations and enhancing consumer acceptance (Castro-Rosas et al., 2017).

The application of nano- and microencapsulation significantly enhances the stability and functional longevity of essential oils and other natural antimicrobials by protecting them against oxidation, volatilization, and light-induced degradation. Encapsulation serves as a physical barrier that shields active compounds from adverse environmental conditions and microbial interference during processing, storage, and handling (Naz et al., 2025). Moreover, these systems enable controlled and sustained release of antimicrobial agents, ensuring that their concentrations remain above the minimum inhibitory concentration (MIC) over extended periods. This prolonged release is particularly advantageous for inhibiting the gradual proliferation of spoilage and pathogenic microorganisms. Importantly, the gradual delivery minimizes abrupt sensory impacts, such as undesirable odors or flavors, that can arise from the direct addition of high concentrations of antimicrobial agents (Mukurumbira et al., 2022).

### 9.3. Edible coatings and films

Recent advances in antimicrobial delivery have highlighted the use of edible coatings and films derived from natural biopolymers, such as chitosan and alginate, particularly for fresh fruits and vegetables. These coatings act as semi-permeable barriers that not only inhibit microbial contamination but also slow respiration and moisture loss, thereby extending shelf life. Derived from brown seaweed, alginate is a biodegradable and biocompatible polysaccharide often used to form hydrogels that are strengthened via calcium ion cross-linking. Incorporating natural antimicrobials such as garlic oil or white ginseng extract into alginate matrices enhances their antimicrobial performance. The gradual release of these agents at the food surface disrupts microbial

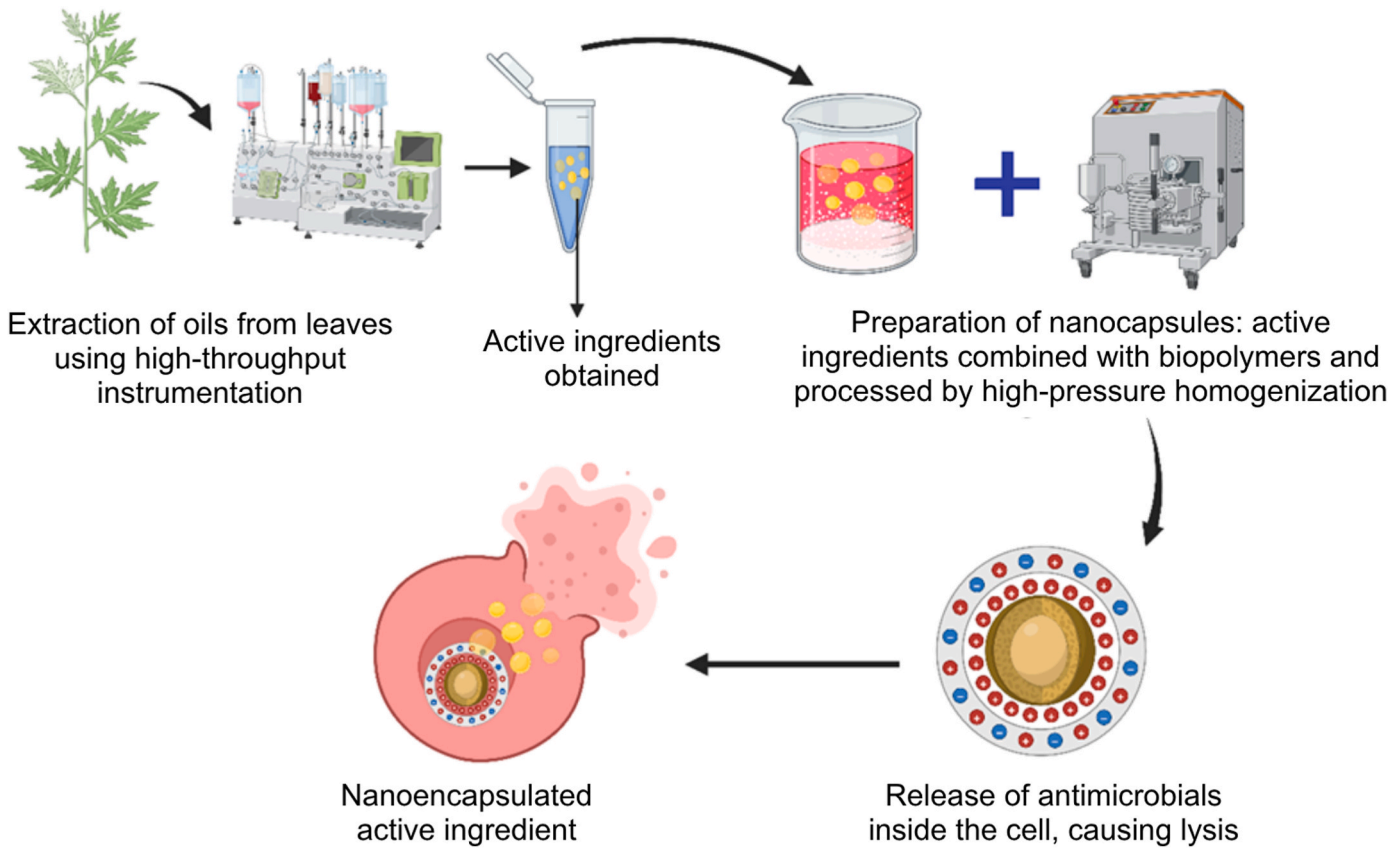


**Fig. 5.** Fabrication of nanoencapsulated plant-derived bioactive antimicrobials. This figure illustrates the sequential process of producing nanoencapsulated natural antimicrobials. Oils are first extracted from plant leaves using high-throughput instrumentation to obtain active bioactive compounds. These are combined with biopolymers and processed via high-pressure homogenization to form stable nanocapsules. The encapsulated antimicrobials provide protection against degradation and enable controlled release. Once delivered into microbial cells, they gradually release active compounds that disrupt cellular activities and induce lysis. This approach enhances the stability, bioavailability, and antimicrobial efficacy of plant-derived compounds while minimizing sensory alterations in food systems.

membranes, inhibits metabolic activity, and suppresses the growth of spoilage and pathogenic organisms including *E. coli* and *L. monocytogenes* (Metha et al., 2024).

Chitosan, a cationic biopolymer produced via chitin deacetylation, exhibits intrinsic antimicrobial activity due to its positive charge. It interacts electrostatically with negatively charged microbial cell membranes, increasing permeability, leading to cytoplasmic leakage and microbial cell death. Additionally, chitosan-based films create oxygen-impermeable barriers that reduce oxidative spoilage. When chitosan is blended with essential oils, tannins, or antimicrobial enzymes such as lysozyme, its antimicrobial efficacy is significantly enhanced (Xing et al., 2016). For instance, chitosan–tapioca starch films enriched with essential oils have demonstrated efficacy against *Lactobacillus* spp. and *Z. bailii* in seafood and bakery products. Moreover, multilayered films incorporating chitosan, tannin, and corn starch offer improved water vapor barrier properties, increased antioxidant capacity, and extended freshness of perishable products such as bananas (Ma et al., 2024).

### 9.4. Active and intelligent packaging

Active packaging refers to packaging systems that actively interact with the food or its surrounding environment to extend shelf life, improve safety, or maintain quality. These systems typically incorporate natural antimicrobial agents that can be gradually released or act as scavengers for oxygen, carbon dioxide, moisture, or ethylene, thereby inhibiting microbial growth and oxidative spoilage (Yildirim et al., 2018). For example, oxygen scavengers reduce oxygen levels in the headspace of packages to limit aerobic microbial proliferation, while antimicrobial films can continuously deliver agents such as essential oils, organic acids, or bacteriocins directly to the food surface. These agents act by disrupting microbial membranes, increasing permeability, inducing leakage of cellular contents, and interfering with metabolic processes, effectively inhibiting spoilage and pathogenic microorganisms including *L. monocytogenes*, *Salmonella* spp., and molds (Rashed et al., 2022).

Polyethylene terephthalate (PET) films embedded with antimicrobial peptides have demonstrated efficacy in suppressing bacterial and yeast growth in perishable products such as dairy and fresh meat, particularly under refrigerated storage. Furthermore, encapsulation techniques employing carriers like halloysite nanotubes to deliver compounds such as thymol or carvacrol enable low-dose, long-acting antimicrobial functionality with minimal sensory impact on food (Kumar et al., 2024).

In contrast, intelligent packaging systems are designed to monitor and communicate the condition of packaged food in real-time. These systems incorporate sensors or indicators that detect changes in temperature, humidity, gas composition, or microbial activity, providing visual or electronic feedback regarding product freshness or spoilage (Dodero et al., 2021). For instance, colorimetric sensors respond to variations in pH or volatile amines produced by microbial metabolism, visibly indicating food deterioration. Additionally, radio-frequency identification (RFID) tags and electronic noses are employed to track product distribution, monitor environmental conditions, and ensure quality assurance throughout the supply chain (Abekoon et al., 2024).

### 9.5. Synergistic formulations and hurdle technologies

Recent advances in food preservation have emphasized the strategic

combination of natural antimicrobials with other agents or technologies to enhance efficacy, mitigate resistance, and extend shelf life. Synergistic formulations leverage the complementary mechanisms of different compounds or treatments, resulting in more effective microbial inhibition at reduced concentrations. For instance, combining organic acids or bacteriocins with essential oils rich in phenolics and terpenes has been shown to produce additive or synergistic antimicrobial effects. These combinations disrupt bacterial membranes, interfere with enzymatic systems, and impair genetic material more efficiently than individual agents alone. Notably, such formulations are particularly effective against resistant strains such as *L. monocytogenes*, *Salmonella* spp., and multidrug-resistant bacteria (Sharma et al., 2024). Moreover, utilizing synergistic blends helps lower the required dosage of each agent, thereby minimizing potential alterations in taste, aroma, and texture commonly associated with single-compound applications.

High-pressure processing (HPP) exemplifies a powerful hurdle technology that, when combined with natural antimicrobials, yields enhanced preservation outcomes. HPP employs pressures exceeding 400 MPa to inactivate microorganisms and enzymes while maintaining food quality attributes. However, some sublethally injured microbes may survive and recover post-treatment. Applying natural antimicrobials such as essential oils, chitosan, or bacteriocins, after HPP effectively suppresses these residual populations, ensuring prolonged safety and stability. This combined approach has proven beneficial in perishable food categories such as meat, dairy, and fresh produce (Chuang & Sheen, 2022). The integration of multiple antimicrobial strategies, whether through chemical synergy or physical treatments, offers a promising route to achieve robust food safety without reliance on synthetic preservatives. Continued exploration of synergistic combinations, supported by mechanistic understanding and sensory evaluation, is essential for designing multifunctional and consumer-acceptable preservation systems.

## 10. Future perspectives and emerging technologies

Despite rapid advancements and promising potential of natural antimicrobials in food preservation, significant research gaps remain. Many antimicrobial compounds derived from plants, microorganisms, and enzymes are still insufficiently characterized, and their efficacy in ensuring food safety has not been comprehensively validated. Further research is needed to isolate, characterize, and catalog these compounds, as well as to determine optimal concentrations that effectively inhibit pathogenic growth without compromising sensory and nutritional quality (Karnwal & Malik, 2024). Although natural antimicrobials are generally perceived as safer by consumers, long-term studies on their health and environmental impacts remain limited (Bensid et al., 2022). In addition, challenges related to scalability, cost-efficiency, and regulatory compliance continue to hinder widespread adoption. Since the efficacy of these compounds can vary depending on the food matrix, consistent performance across diverse systems must be ensured. Addressing these limitations is essential to enable a successful transition from synthetic to natural preservatives in sustainable food preservation (Karnwal & Malik, 2024).

### *10.1. Novel extraction technologies*

Conventional extraction methods often rely on organic solvents, high temperature, or long processing times, which may degrade heat-labile bioactives, reduce yields, and increase environmental burden. To overcome these drawbacks, novel approaches such as ultrasound-assisted, microwave-assisted, enzyme-assisted, and supercritical fluid extraction have been developed. These techniques improve yield and preserve functional integrity, while also reducing solvent use and energy consumption (Sat et al., 2023; Karnwal & Malik, 2024). Such methods are particularly promising for tapping underutilized sources, including plant by-products, fungi, and marine resources, thereby broadening the diversity of antimicrobial compounds available for food applications. Increasing efficiency and sustainability in extraction is foundational for scalable production that meets regulatory and clean-label demands (Fan et al., 2024).

### *10.2. Omics-based discovery of new antimicrobials*

Omics technologies, genomics, transcriptomics, proteomics, and metabolomics have revolutionized the identification of natural antimicrobial candidates. These methods enable high-throughput discovery of antimicrobial genes, proteins, and metabolites, even in organisms previously considered unculturable. Beyond identification, omics approaches help elucidate molecular mechanisms of action and interactions within microbial communities. The integration of omics data with bioinformatics and machine learning further enhances the ability to predict activity, optimize targets, and assess safety profiles (Dutta et al., 2022). By accelerating discovery and characterization, omics-driven approaches will play a pivotal role in building a systematic pipeline for next-generation natural antimicrobials (Mahato et al., 2024).

### *10.3. Encapsulation and nanotechnology for stability*

One of the major challenges for natural antimicrobials is instability and strong sensory impact. Encapsulation and nanotechnology offer solutions by protecting sensitive compounds such as essential oils and polyphenols from degradation caused by heat, light, and oxygen. Nanoencapsulation also improves solubility and bioavailability of hydrophobic antimicrobials, enabling controlled release that prolongs antimicrobial activity while minimizing undesirable effects on food flavor and aroma (Delshadi et al., 2021; Lelis et al., 2021). These advanced delivery systems are still in early stages of industrial adoption but have strong potential to extend shelf life and maintain food quality under clean-label formulations.

### *10.4. Consumer acceptance and sensory impact*

Consumer perception remains a decisive factor in the success of natural antimicrobial strategies. While many natural antimicrobials are highly active, compounds such as essential oils and phenolics can impart intense flavors or aromas that alter the original sensory profile of foods (Quinto et al., 2019). Encapsulation and controlled-release systems can mitigate these effects, yet balancing antimicrobial efficacy with acceptable sensory qualities remains a key challenge (Teixeira et al., 2022). Continuous consumer testing and sensory optimization will be critical to achieve products that meet both safety requirements and market expectations. Ultimately, technologies that harmonize food safety with sensory quality will have the greatest likelihood of consumer acceptance and commercial success (Kocić-Tanackov & Pavlović, 2023).

### *10.5. Market outlook and opportunities*

Global consumer demand for safer, cleaner, and minimally processed foods is fueling the market for natural antimicrobials. The global market for natural and plant-based antimicrobial agents was valued at approximately USD 2.9 billion in 2024 and is projected to grow at a compound annual growth rate (CAGR) of over 5 % through 2034 (Shi et al., 2024). Food manufacturers are increasingly investing in Research and Development to integrate natural antimicrobials into novel packaging systems and formulations that extend shelf life while meeting clean-label expectations. Nevertheless, scalability, regulatory approvals, and sensory optimization remain hurdles. Collaborative efforts among researchers, industry stakeholders, and regulatory bodies will be essential to unlock the full potential of natural antimicrobials and accelerate their translation from laboratory research to real-world food systems.

## 11. Conclusion

Natural antimicrobials have emerged as effective solutions to meet the growing consumer demand for clean-label food products by ensuring microbial safety and extending shelf life. They exert their effects by disrupting microbial cell membranes and interfering with key metabolic pathways. In addition, many natural antimicrobials possess antioxidant properties that help maintain food quality without significantly altering its sensory characteristics. Most natural antimicrobials are classified as GRAS and are derived from renewable resources, supporting both environmental sustainability and the reduction of synthetic preservative use. However, several challenges remain, including the impact of food processing conditions on antimicrobial efficacy and potential alterations in flavor and sensory attributes. Scaling up production while maintaining consistency and complying with diverse international regulations also presents significant obstacles. To fully harness the potential of natural antimicrobials, continued research is essential to optimize synergistic formulations, elucidate their mechanisms of action, verify safety, and advance delivery systems such as nanoencapsulation and active packaging. Harmonized regulatory frameworks and stronger international guidelines will facilitate their broader adoption, promoting the development of safe, natural, and sustainable food preservation strategies aligned with both industry and consumer expectations.

## CRediT authorship contribution statement

**Anand Kumar:** Writing – original draft, Formal analysis. **Suprativ Das:** Writing – original draft, Methodology. **Sadaqat Ali:** Writing – original draft, Software. **Swapnil Ganesh Jaiswal:** Writing – review & editing, Formal analysis. **Ahmad Rabbani:** Writing – review & editing, Formal analysis. **Syed Mohammad Ehsanur Rahman:** Writing – review & editing, Formal analysis. **Ramachandran Chelliah:** Writing – review & editing, Formal analysis. **Deog-Hwan Oh:** Writing – review & editing, Formal analysis. **Shucheng Liu:** Writing – review & editing, Supervision, Conceptualization. **Shuai Wei:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare no conflicts of interest.

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## Data availability

No data was used for the research described in the article.

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