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Active packaging coating films based on natural bioactive compounds

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Abstract

Food storability and quality can be affected by a wide range of factors, including physiological, natural, and microbiological ones. These include heat, oxidation, chemicals, yeast, pathogens, and microscopic organisms. As the previously mentioned arguments suggest, innovative food packaging materials may be able to meet consumers' needs and satisfy their desire for prepared foods with extended shelf lives that are both realistically useful and of high quality. Active packaging coating films are promising systems to be used as active ingredient carriers, due to their dual classification as food and packaging components, they must meet some specifications, including low raw material and processing costs, good sensory qualities, high mechanical and barrier qualities, biochemical, physical-chemical, and microbial stability, safety, and non-polluting nature. Achieving these goals of maintaining food quality, safety, and sensory attributes is made possible by the use of bioactive substances and additives. To make films and coatings materials well-processed and efficient in practical, everyday food systems, research efforts are concentrated on optimizing their composition. The materials used in antimicrobial food packaging could comprehend the future needs of these customers. The materials used to package food were designed to either interact with the food itself or with the environment in which it is placed. The key advancements in the field of innovative food packaging have been attributed to the impact of these active agents on consumer health. In keeping with this, the survey paper provides an overview of the latest research on the development of antimicrobial food packaging materials.

Keywords: Active packaging films, Natural bioactive compounds, Multifunctionality.

1. Introduction

The spoilage and food deterioration is a critical issue that is mainly caused by many foodborne pathogens. These pathogens mostly grow on food surfaces and also some microorganisms grow in polymers' volume in the pores and gels [1, 2]. A reasonably novel packaging technology has emerged which, based on the interaction between foods and packages termed as active packaging [3]. These active packaging could be utilized as alternatives to conventional food packaging materials for example, refrigeration, freezing, drying, fermentation, and thermal processing [4], and the usage of organic acids and salt additives to extend food product's shelf life [5-7].

Active packaging has several different functions, including Oxygen/ethylene scavengers, carbon dioxide absorbers/emitters, flavor emission, moisture scavenging, and antimicrobial and antioxidant action [8, 9] as in Figure (1 & 2).

By directly coating food or film candidates with naturally derived or synthetic antimicrobial agents, antimicrobial packaging prospects are probably one of the most promising packaging categories [10].

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Figure (1): Active agents for food packaging functions [11].

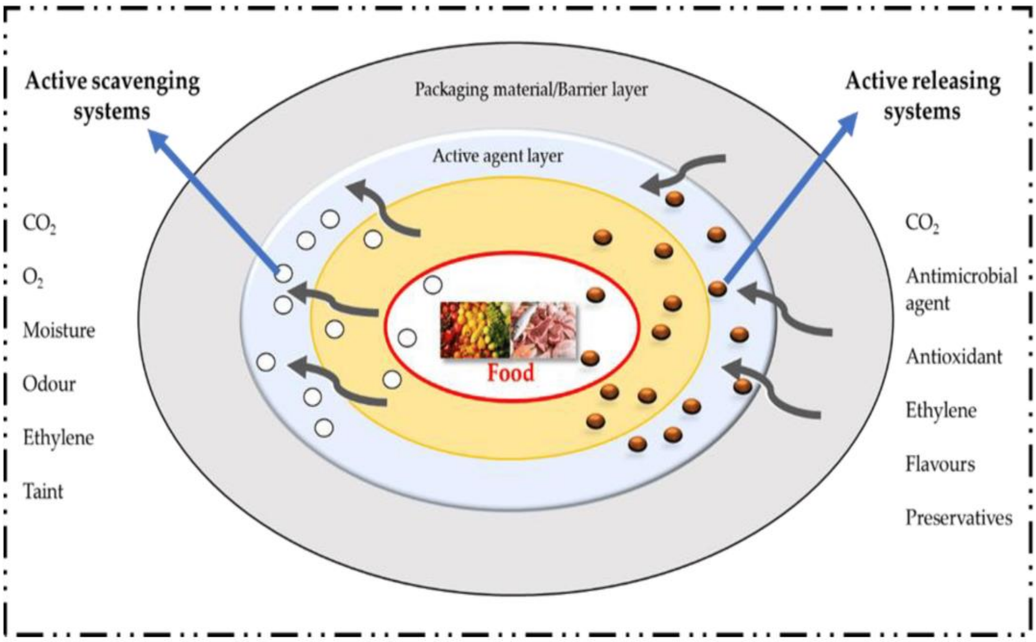

Figure (2): The mode of action of active packaging materials against the food spoilage caused organisms through Active scavenging and releasing process [7].

Antimicrobial agents have some drawbacks that restrict their effectiveness. For example, typical antimicrobial agent applications, such as dripping or spraying on the food surface, have no tangible impact due to their quick diffusion and interactions with food [12]. Furthermore, the taste of the food is severely affected by these chemicals [13].

Foodborne bacteria growth is inhibited by the antimicrobial agents added to polymer matrix materials during their interaction with the polymer surface. Maintain a sufficient and continual presence of antimicrobial agents on the food surface by controlling the antimicrobial agents' rate of diffusion [14, 15]. Thus, to create actively antimicrobial food packaging, antimicrobial chemicals are frequently included in film composites.

Food packaging's antibacterial capabilities may depend on antimicrobial agents, which can be categorized as inorganic antimicrobial, organic, and synthetic agents depending on their resources. It is simple to generate comparatively non-toxic antibacterial agents [16, 17].

The types of original animals or plants that may be rare or expensive determine how many naturally-occurring antimicrobial agents there are. The development of food manufacturing and the need for natural antibacterial agents and packaging materials typically result in limited supply. As a result, synthetic antibacterial agents, either organic or inorganic, have been developed [18, 19].

Conventional food packaging should benefit from the easy production of common polymeric films that have good performance [20]. The best polymeric materials for food packaging protection offer outstanding mechanical and physical qualities, including strength, stiffness, moisture, and oxygen barrier, and the ability to shield food from microbiological perforation [21, 22]. Therefore, the purpose of this review paper is to focus light on some study findings on the use of naturally occurring antimicrobial agents that are used as innovative antimicrobial packaging materials based on polymeric films.

2. Natural occurring antimicrobial substances

2.1. Essential oils

The naturally occurring oily compounds known as essential oils (EOs) are produced as metabolites by various plant parts, including seeds, buds, flowers, leaves, roots, fruits, twigs, and bark [23]. The majority of essential oils are blends of naturally

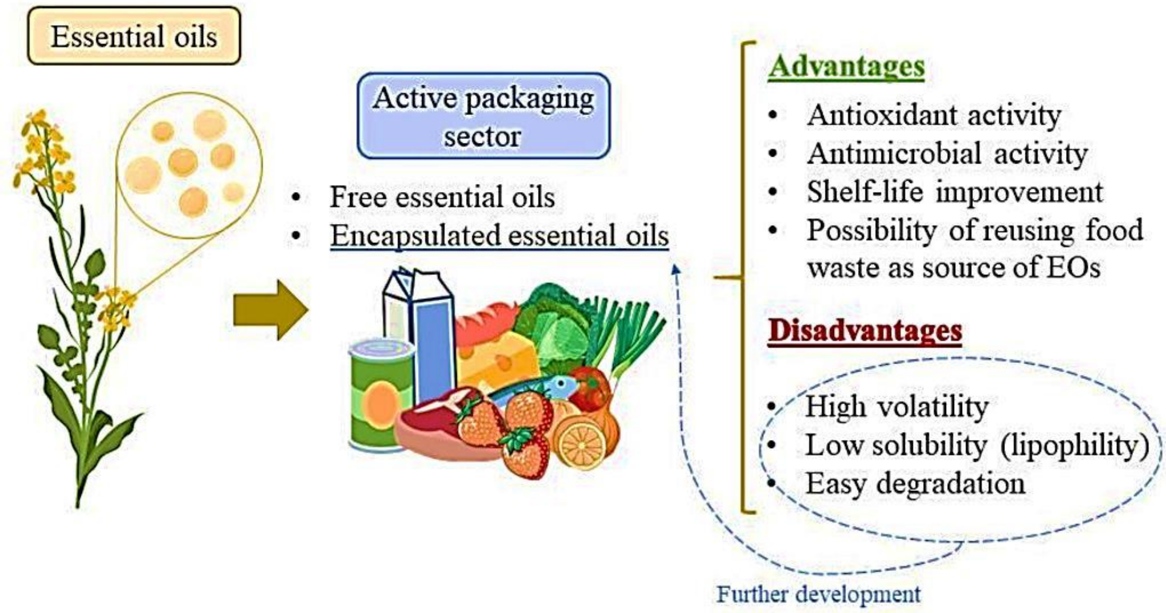

Figure (3): Advantages and disadvantages of essential oils (EOs) activity in the food active packaging field [24].

occurring volatile substances that are extracted from plant parts through compression or distillation [25-27]. Alkaloids, flavonoids, isoflavones, monoterpenes, phenolic acids, carotenoids, and aldehydes are the primary constituents of essential oils [28].

Essential oils are lipophilic, volatile, and nearly water-insoluble substances [29]. In addition, the Food and Drug Administration states that antimicrobial agents have antioxidant, antiseptic, and antiparasitic characteristics among their many possible attributes, in addition to their activity against fungi and viruses. As a result, essential oils are now considered safe (GRAS) [30]. The exceptional biological performance of essential oils renders them the most widely used additives in the food manufacturing industry. Therefore, essential oils could be used to extend the shelf life of food as shown in figure (3).

To use essential oils as antimicrobial agents, three methods are used: coating food packaging with essential oils [31], loading essential oils into an antibacterial pouch, and incorporating essential oils into polymeric matrices. In general, mixing essential oils into polymer matrices is a convenient and straightforward process for the mass production sector. Edible films made from fruits and vegetables are widely used worldwide, and essential oils are blended with biodegradable polymers like PLA, chitosan, and gelatin [32-36].

In this regard, some essential oils, like oregano, lemongrass, and vanillin, worked well in edible coatings constructed from alginate and pureed apples. By reducing the softening and ripening reactions of coated fresh-cut apples, this modified coating increases the shelf life of fresh apples. Apple puree-alginate coatings containing calcium chloride and N-acetylcysteine, an anti-browning agent, are essential for preserving firmness and color. Apple pieces inoculated with *Listeria Innocua* appeared to be effectively inhibited from growing by a successful coating that included essential oils. The color and texture of the coated fresh-cut apples are significantly influenced by lemongrass [37].

Methyl ester of cinnamic acid, the primary volatile compound found in strawberries, has been shown to have antifungal properties. They are created and then released when the fruit reaches maturity. Methyl Cinnamate and carvacrol, the primary ingredient in oregano and thyme essential oils, were added to strawberry puree. The shelf life of strawberries was extended by carvacrol vapors emitted from the edible strawberry puree film [38]. For the production of antimicrobial films, PLA is coated with varying concentrations of cellulose nano particle (NC), *Mentha piperita* EO (MPO), and *Bunium persicum* EO (BPO) [39]. For 12 days, the ground beef was kept at 4 ^{o}C after being tightly sealed with these modified films. Following the evaluation of these films, it was found that Mentha piperita EO (MPO) and *Bunium persicum* EO (BPO) exhibited antimicrobial activity against *Pseudomonas*, *Staphylococcus aureus*, and *Enterobacteriaceae*. The performance of the water vapor barrier is enhanced by the addition of essential oils. Certain morphological parameters had been changed.

It is known that copaiba oil has antibacterial properties against Bacillus subtilis. Paper, polylactic acid film, and copaiba oil were combined. Up to a 20-weight percent concentration of copaiba oil, the modified paper and polylactic film exhibited a characteristic inhibition zone against *Bacillus subtilis* [8]. A polylactic film was prepared with varying concentrations of *Origanum vulgare L.virens* essential oil (OEO) [40]. The novel packaging films demonstrated antimicrobial activity against molds and yeasts up to 5% and 10% of *Origanum vulgare L.virens* essential oil concentrations when evaluated in terms of their antimicrobial activity for fresh salads. With a few minor modifications, the new active food packaging film has appropriate mechanical and physical features.

The barrier, physical, antioxidant, antibacterial, and thermal properties of the films were examined after they were treated with 1% Quince seed mucilage (QSM). *Salmonella typhimurium* and *Pseudomonas aeruginosa* are not susceptible to the modified films' antibacterial properties. However, it significantly prevents *S. aureus* and *E. coli* from growing [41].

The effect of directly adding copaiba oil and microencapsulating it in films composed of *Xanthosoma mafaffa* Schott starch. Copaiba oil was first evaluated for its antibacterial efficacy against both gram-positive and gram-negative bacteria using gas chromatography combined with mass spectrometry (GC-MS). Copaiba oil was primarily composed of sesquiterpenes, primarily β-caryophyllene, which had antibacterial action against both *B. subtilis* and *S. aureus*. The gram-positive bacteria tested were inhibited by films incorporated with microparticles, forming inhibition zones. This suggests that encapsulating copaiba oil was more effective in protecting bioactive compounds from the oil, and that mangarito starch-based films incorporated with copaiba oil could be used as biodegradable packaging [42].

Films composed of sweet potato starch and montmorillonite (MMT) nanoclays were produced with thyme essential oil (TEO) present [43]. This biodegradable, active nanocomposite film demonstrated that starch/clay films infused with thyme essential oil could control the growth of *Salmonella Typhimurium* and *Escherichia coli*, thereby preserving the quality of baby spinach leaves during refrigeration. Additionally, MMT improved the mechanical properties of the starch films. The antibacterial properties of thyme essential oil (TEO) were examined through its embedding into biodegradable skate gelatin (SSG) films. According to the findings, TEO (1%) effectively inhibits the growth of *L. monocytogenes* and *E. coli* in the samples of packaged chicken [44].

Researchers have reported the essential oil of rosemary as a potent antibacterial agent. it has been incorporated into chitosan films and it has been used in food preservation [45]. This study demonstrated that chitosan-modified films exhibited greater antibacterial activity than pure chitosan films.

Non-biodegradable polymers are combined with essential oils to facilitate manufacturing and processing. As a result, there are still certain possible shortcomings with traditional processing methods, which could give rise to the development of new, innovative methods. Thus, one of the most effective methods for creating nanofibers is electrospinning.

The concept behind the electrospinning process was the application of an external electric field, which allowed the polymer solution or melted polymers to produce nanofibers with diameters in the sub-micron range. This method is distinguished by many benefits, including highly porous structures and large surface area. Using the electrospinning technique to encapsulate tea tree oil (TTO) and beta-cyclodextrin (β-CD) into poly (ethylene oxide) (PEO) was inspired by the many benefits of using this technique to produce food packages [8, 46]. For seven days, *E.coli* was used as a test subject for the antibacterial activity of TTO-(β-CD) polyethylene oxide electrospun treated with cold plasma at varying temperatures. The antibacterial characteristics of the test showed a significant increase following the plasma treatment, and at 4 or 12 ^{o}C, an inhibition effectiveness of 99.99% was reached, indicating that the beef can be protected by TTO-(β-CD) polyethylene oxide electrospun. Electrospun polymeric oxide-cinnamon (CEO)/β-cyclodextrin (β-CD) proteoliposomes have been generated. Throughout the proteoliposomes' protein proteolysis, the CEO-controlled release occurred. When applied to beef samples, this electrospun has shown a strong antibacterial effect against B. cereus [47, 48]. Protein fragments with specific functions, such as antioxidant, antimicrobial, anti-hypertensive, and antithrombotic properties, are known as antimicrobial peptides, and they include bacteriocins [49]. These benefits to consumers' health are cumulative.

Liposomal encapsulated natural antimicrobials have been the subject of very few studies that provide insight into their use in food packaging. The antibacterial efficacy of pectin film comprising liposome-encapsulated clove oil, garlic oil, and pomegranate extract was reported. When compared to pectin film containing antimicrobial extract without encapsulation, they demonstrated improved antibacterial activity of the film having encapsulated antimicrobials. Phospholipids like lecithin and milk fat globule membrane phospholipids are typically utilized to create liposomes that are employed to encapsulate different functional substances like medications and nutritional supplements [50].

2.2. Cinnamaldehyde

Significant research has been conducted in the last ten years on natural ingredients produced by herbs that possess strong antibacterial and antioxidant capabilities [51]. Cinnamaldehyde is a naturally occurring chemical derived from plants that has long been used as an additive and flavoring in food preparation.

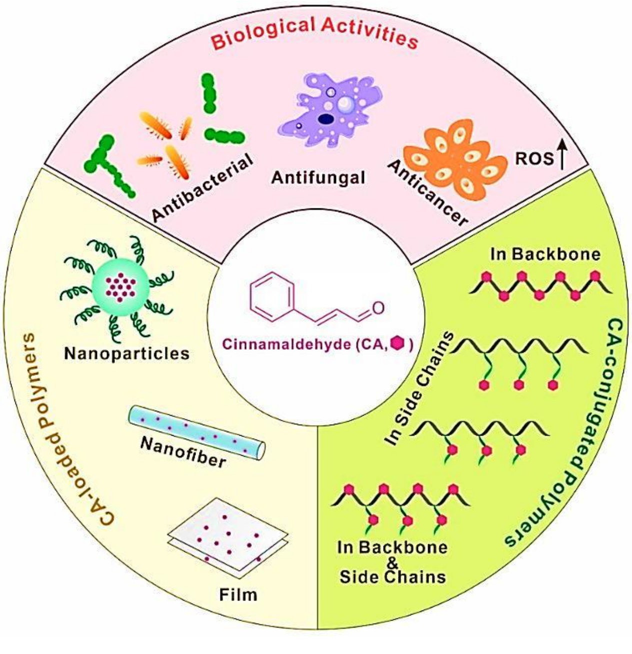

Figure (4): Cinnamaldehyde-contained polymer biological activities activity [52].

Along with Flores, Pelegrín, Ramos, Jiménez, & Garrigós (2021); Karim, Fathi, & Soleimanian-Zad (2021); Makwana, Choudhary, Dogra, Kohli, & Haddock (2014), cinnamaldehyde exhibits antibacterial, antioxidant, antidiabetic, and anticancer properties [50, 53, 54].

Cinnamaldehyde is an unsaturated aldehyde and phenolic terpenoid that is abundant in cinnamon and is often used as a food ingredient. Its antibacterial, antifungal, anti-inflammatory, and antioxidant qualities are noteworthy [55] as in Figure (4).

It is commonly used in the food, cosmetic, and pharmaceutical industries due to its inherent antibacterial properties [55-58].

It has also been suggested that CIN inhibits the enzymes quitin synthase 1 and β -(1,3) glucan synthase, both of which are essential for the synthesis of enzymes in the interior membranes of many bacteria, including *Staphylococcus aureus* and *E. coli*, as well as the cell walls of yeasts and molds [59]. Several investigations have demonstrated that CIN can be used in a range of concentrations as a potential antibacterial agent [50].

A liquefied ball-milled shrimp shell chitin/polyvinyl alcohol (LBSC/PVA) blend film was created as a biodegradable packaging material. It had cyclodextrin/cinnamaldehyde (CD/CIN) inclusions. Host-guest interactions effectively enclosed CIN in the CD cavity, leading to a significantly improved prolonged release of CIN. The inclusion of CD/CIN significantly enhanced the blend films' performance in terms of food preservation and antibacterial activity. Furthermore, compared to the 3 wt% CIN/LBSC/PVA blend film, the 3 wt% CD/CIN /LBSC/PVA blend film showed noticeably longer antibacterial activity and better cherry tomato preservation performance, underscoring the critical role of -CD in delaying CIN release [60].

Fish gelatin antibacterial composite films were made using cinnamon aldehyde (CIN) and its sulfobutyl ether-β-cyclodextrin inclusion complex (CIN/S), according to Jiulin Wu et al. (2023). The addition of (CIN/S) enhanced stretchability and function as a light barrier. The film-forming solution with CIN and CIN/S demonstrated the best inhibitory ratio against *Pseudomonas aeruginosa*; it was 98.43 1.11% in the first period and remained 82.97 4.55% at 72 hours. Furthermore, the gelatin packaging solution effectively inhibited microbial growth while maintaining the preservation of grass carp slices by using CIN and CIN/S. At the end of storage, total volatile salt-based nitrogen (TVB-N) is less than 10 mg/100 g indicating that the active coating may certainly extend the shelf life of fish muscle [61].

Shoumei Wan et al. reported high amylose corn starch-cinnamaldehyde (HACS-CIN) inclusion complex antibacterial films (2023). With an encapsulation efficiency of up to 39.19%, the releasing rate tests showed that HACS-CIN inclusion films could lower the rate of CIN volatilization. The films showed remarkable mechanical properties with an elongation at a break of 44.95% and a tensile strength of 14.77 MPa. Additionally, they were transparent, with a 70% visible light transmittance. The UV transmittance test showed good UV-blocking properties with 30% UV light transmittance. Antibacterial tests showed that the growth of E. Coli and S. aureus was inhibited [62]. A study of zein film loaded with essential oils (EOs) was reported by another investigation. To give the films antimicrobial properties, carvacrol, cinnamaldehyde (CIN), and eugenol were added; polyethylene glycol (PEG) and oleic acid (OA) were chosen to enhance the mechanical properties. The findings demonstrated that, in comparison to OA, PEG effectively increases the tensile strength and elongation (%E) of zein films; however, PEG also enhanced the films' water barrier., the zein film embedded with EO exhibited superior antimicrobial activity compared to EO alone. The produced films have a high potential for use as active food packaging because they exhibit a gradual release of essential oils and antimicrobial effects for up to 96 hours [63]. An additional investigation investigated the reversible insertion of cinnamon aldehyde via imino-covalent bonding into chitosan films. Chitosan-Schiff base films' antibacterial capabilities were examined in vitro against Listeria monocytogenes-inoculated milk, Staphylococcus aureus, and Escherichia coli. Depending on the methodology used, different levels of imino bond hydrolysis and the subsequent release of cinnamon aldehyde were obtained, which in turn affected the antimicrobial activity. The growth of *L. monocytogenes* was inhibited for 12 days under freezing conditions for Schiff base-chitosan derivative films that were exposed towards varying temperature / time treatments. This may increase the biological shelf life of such foodstuffs. The results of the milk's sensory study in relation to the films indicate that potential customers are not put off by the cinnamon aroma. These innovative films may find application in the development of antimicrobial food packaging as well as in other technological fields requiring sustained-release mechanisms [64]. Zhang et al. (2021) treated a Kraft paper with acetylated cellulose solution containing varying amounts of cinnamon aldehyde (CIN) to create a robust, antibacterial, high-barrier paper. Investigations were conducted on coated paper's mechanical, barrier, antibacterial, and antioxidant characteristics. The treated paper demonstrated outstanding antibacterial activity against Staphylococcus aureus and Escherichia coli and antioxidant activity when coated with 6% v/v CIN. Notably, after coating, the paper's dry and wet tensile strengths increased by 26.4 and 10.6 MPa, respectively. Additionally, there was a significant improvement in the coated papers' barrier aspects against oxygen, water vapor, oil, and water, particularly concerning the water barrier rate, which reached 96.4%. Our coated papers with more than 6% v/v CIN could raise the minimum shelf life of beef [65].

2.3. Bacteriocins

Bacteriocins are a kind of peptide produced by ribosomes and excreted extracellularly by gram-positive and gram-negative bacteria [66-68]. Bacteriocin has been chosen as a potential medicinal candidate to replace chemicals and antibiotics in the future due to its lower toxicity and proteinaceous nature. Furthermore, antibiotics used to get rid of infections may have a deleterious impact on the gut microbiota since they kill both the targeted and adjacent microbial populations [69-72].

The two main types of bacteriocins produced by lactic acid bacteria are modified peptides known as [lanthionine- containing bacteriocins], which include pediocin and sakacin, and [non-lanthionine-containing bacteriocins], which include nisin, azoline, linaridin, glycocin, cyanobactin, sactiobiotic, lasso peptide, and thiopeptide. Among the commonly used bacteriocins are nisin and pediocin [73]. Bacteriocin is a naturally occurring antimicrobial peptide that bacteria produce to defend against pathogens or other bacteria by either inhibiting or removing them without causing harm to themselves.

Lactic acid bacteria (LAB) and other bacteria, containing strains of Bacillus, Staphylococcus, and Escherichia coli, are typically the sources of bacteriocin. LAB is a class of Gram-positive bacteria that are highly tolerant of acidic environments. They are non-spore forming, can be rod- or spherical-shaped, and have similar physiology and metabolism [74]. Bacteriocin-producing bacterial strains can help incorporate bacteriocins into food during cultivation, or they can be added directly to food. Recently, bacteriocins have attracted extensive attention in the healthcare sector as antibacterial and anticancer agents [75] as in figure (5).

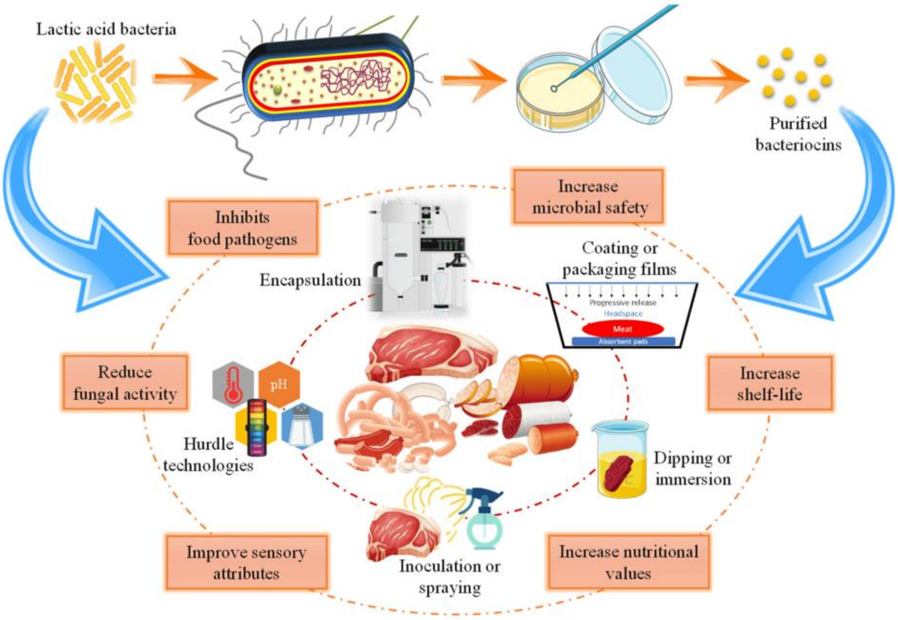

Figure (5): Biopreservation effect and bacteriocins of lactic acid bacteria on packaging quality of meat products [76].

Many bacteriocins, like nisin, have demonstrated exceptional and targeted antimicrobial efficacy against multi-drug resistant (MDR) gram-positive bacterial strains, including methicillin-resistant Staphylococcus aureus (MRSA).

Furthermore, bacteriocins which are extensively studied in foods produced by gram-positive bacteria can naturally resist the action of gram-negative bacteria. It is important to remember that gram-positive bacteria can impede the growth of gram-negative bacteria like *P.aeruginosa* by combining their bacteriocins with antibacterial nanoparticles. According to Vukomanović et al. (2017), Au-nisin has successfully inhibited the growth of *P.aeruginosa* and *E. coli* bacteria [77]. According to Bandara, Korpole, and Grover (2018), there have been positive results from numerous anticancer studies where researchers are currently examining the biomedical properties of bacteriocins [78].

Consequently, bacteriocins are of great interest to researchers as safe materials for biomedical applications [9, 79]. According to a study reported by Contessa et al. (2021), a bioplastic film was created using naturally occurring substances like agar and bacteriocin that were extracted from a food matrix by *Lactobacillus sakei.* mechanical, barrier, and physical features in addition to good handling characteristics. The film was then used in a commercial curd cheese product that has a high perishability and its microbiological stability was monitored. The film demonstrated good barrier qualities with the external environment and appropriate migration of the antibacterial ingredient to the product, supporting less food contamination [80].

In an intriguing investigation, the viability and antibacterial activity of bacteriocin-producing lactic acid bacteria (LAB) embedded into whey protein/inulin/gelatine (WP) edible films in the presence or absence of nutrition (modified MRS broth) were studied. In an in vitro system as well as on cooked ham, films containing nutrients showed good antibacterial efficacy against *L.innocua* C6. The incorporation of LAB has a substantial effect on the film structure: it decreases the level of viscosity of the film-forming solution and increases elasticity and percentage elongation. Water vapor transfer rate and solubility, on the other hand, had no influence. Thus, in the presence of modified MRS broth, WP-based films can be employed as efficient transport and carrier systems for lactic acid bacteria to produce bioactive edible films or coatings with antimicrobial capabilities [81].

Nisin is an antibacterial peptide formed by *Lactococcus lactics* and lactic acid bacterium [75, 82]. It is the most widely used bacteriocin in active food packaging. Nisin could prevent Gram-positive microorganism growth for example *Listeria monocytogenes* [83-85] and stop spore germination [86]. Nisin contains both hydrophobic groups and positive charges. The antibacterial mechanism mode of nisin was described by the electrostatic interaction that occurred between the positively charged peptide and negatively charged bacteria plasma membrane, enabling attachment of the peptide to the bacterial cell [87]. Additionally, the hydrophobicity of amino groups of peptide and bacterial plasma membrane could play a significant role in changing the bacterial plasma membrane`s permeability and accordingly facilitate the leak out of the cellar components such as DNA and causing bacterial cell death. To create antimicrobial film candidates, nisin could be easily incorporated into polymers by mixing with polymers or coating on the film to obtain food packaging polymer candidates. Many investigations have reported nisin-modified packaging film adequacy to avoid the growth of various foodborne microbes. Maresca and Mauriello (2022) constructed an antibacterial packaging film by combining nisin directly with cellulose nanofibers (CNF), and then coating it into polylactic acid (PLA), polypropylene (PP), and polyethylene (PE) sheets. CNF-Nisin+PLA linked films inhibited the development of Listeria *innocua* 1770 during meat product preservation. In vitro, the films were efficacious against the indicator bacteria *Brochothrix thermosphacta* and *Listeria innocua.* Furthermore, when the hamburgers were held in active films for two days, the Listeria population was reduced by roughly 1.4 log cycles [88].

Many reports have pointed to the effectiveness of combining three different antimicrobial ingredients, such as a starch film-based food packaging film that was engineered by incorporating nisin, lysozyme, and EDTA [89, 90]. The findings suggest that nisin and EDTA have a limited synergistic effect on *Listeria monocytogenes* of ultra-filtered (UF) cheese packages compared with pure chitosan film. While; the films modified with nisin only have a significant inhibitory influence on *L. monocytogenes.* When the ultra-filtered cheese was sealed with chitosan-nisin composite film and stored at 4 ^{o}C, for 14 days, showed no deterioration upon exposure to *L. monocytogenes*. Nisin can kill microorganisms and keep food fresh even at room temperature. This fact was investigated by applying nisin to a popular Korean food called seasoned [91]. Seasoned beef was stored at a low temperature (4 ^{o}C) for 60 days and at room temperature at 25 ^{o}C. The results showed a little growth of mesophilic microbes of the seasoned beef packaged with nisin, whereas, the seasoned packaged without nisin has a remarkable increase in microbial growth. This indicates that nisin has a powerful antimicrobial effect even at ambient temperature. Additionally, nisin not only has a shelf-life prolonging contribution but also; enhances the mechanical properties of packaging films. Investigation of nisin release rates is a critical issue for food preservation, the nisin release rates were studied in sodium caseinate, hydroxypropyl methylcellulose (HPMC), chitosan, and PLA films at the temperature of 4 and 40 ^{o}C in a water/ethanol solution [92, 93]. They found that the temperature has a positive relationship with release rates; while HPMC exhibits the highest nisin diffusion coefficient and chitosan follows the reverse trend.

Nisin could be incorporated into cellulose to fabricate green and good mechanical and physical films of antimicrobial activity. This film was applied to meat and exhibited a reduction in the growth against *L. monocytogenes* after 14 days of storage [94]. Some comparative investigation on cellulose-fiber-based containing nisin was conducted to be used as antimicrobial packaging. Two different routes of utilizing nisin, the first is the direct mixing of nisin with basic food packages and the second is the fabrication of convenient and green nisin-grafted carboxylated cellulose Nano fiber films [95]. They found that the grafting route exhibits a powerful antimicrobial activity against a diverse Gram-positive bacterium, whereas; the mixing route with cellulose Nano fibers causes inhibition of *B. subtilis* growth. These results explain that the more convenient mixing route retains an antimicrobial effect shorter time than the grafting procedure.

Nisin-based films can be applied in processed meat preservations; nisin could be incorporated into synthetic biodegradable polymers for example; polyhydroxybutyrate (PHB) and polycaprolactone (PCL) film was developed [96]. This film exhibits an inhibition effect against *Lactobacillus plantarum* existing on the sliced ham. In another investigation; the polyvinyl alcohol-nisin-based film exhibits reduced levels of *Listeria monocytogenes* on sliced fermented sausages without sodium salt [97]. They found that the processing treatment has a significant effect on antimicrobial properties and gives a new guide for antimicrobial food packaging.

Pediocin is an unmodified small protein with stable physicochemical properties, thermal stability, and broad-spectrum antimicrobial properties. Pediocin is produced by *pediococcus acidilactici.* Pediocin has high temperature and pH adaptability. Due to the polypeptide nature of pediocin, it can be degraded in the human body. It can efficiently inhibit diverse foodborne bacteria. Thereby, pediocin can be industrialized as a new generation of natural food preservative materials [98, 99]. The cellulose-pediocin-based films could be utilized in processed meat preservation as described in many investigations in which the ham slices were stored for up to 15 days. The findings showed those films of 25% and 50% pediocin have an effective antimicrobial activity against many pathogenic foodborne bacteria [100]. In other studies, Nanocomposite-pediocin films could be utilized in food protection; Nanocomposite films containing pediocin and ZnO Nanoparticles showed antimicrobial properties against *Staphylococcus aureus* and *Listeria monocytogens* [8]. However, pediocin showed a significant effect on the mechanical properties of films, it results in less film stiffness and enhanced film elasticity. In some cases, crude pediocin is unable to prohibit microbial growth; these findings were investigated by modifying poly hydroxyl butyrate (PHB) with crude pediocin antimicrobial [101]. The results showed the antimicrobial activity of polyhydroxyl butyrate against pathogenic, spoilage bacteria and fungi. Whereas; crude pediocin alone is unable to prevent the fungal growth, further demonstrating the synergistic effect of pediocin and polyhydroxy butyrate films favor antifungal activity. Regardless, nisin is still the merely naturally-bacteriocins food preservative and it is the more acceptable bacteriocins by the Joint Food Additives Committee (FAO/WHO). Although pediocin safety has been understood, it will be a more prospective natural preservative than nisin and will be a wide commodity.

3. Enzymes

Enzymes such as lysozyme [102], glucose oxidase [103], lactoferrin [104], or the lactoperoxidase system can be taken advantage of as potent antimicrobials in food packaging through chemical binding, grafting, or physical trapping in packaging materials [105-108].

Lysozyme is one of the most commonly utilized enzymes as a preservation agent for food due to its established antibacterial activity against bacteria, fungi, protozoans, and viruses [109, 110]. This enzyme, however, is more efficient against *Gram-positive bacteria* owing to its capacity for breaking down the glycosidic linkages of peptidoglycan in these bacteria's cell walls [111, 112]. For this reason, lysozyme is occasionally combined with various other enzymes or substances, like lactoferrin or EDTA, in the packaging [113, 114]. To enhance the antibacterial activity of lysozyme against *Gram-negative bacteria,* it is combined with lactoferrin. Lactoferrin, a whey protein that combines ferric ions, works against microorganisms by depriving them of iron and changing the permeability of *Gram-negative bacteria* through its interaction with components of lipopolysaccharides [115]. Through the oxidation of β-D-glucose, the enzyme glucose oxidase (GO), a flavoprotein isolated from many fungal species, particularly *A. niger, and Penicillium* species, catalyzes the generation of gluconic acid And hydrogen peroxide, which shows its antimicrobial action [9]. So far, this enzyme has demonstrated effective antibacterial activity against pathogenic foodborne bacteria such as *Staphylococcus aureus, Salmonella infantis, Bacillus cereus, Clostridium perfringens, Listeria monocytogens,* and *Campylobacter jejuni* [116].

Recent studies have looked into lysozyme immobilization approaches such as physical mixing and chemical bonding. Among the experiments is the use of a PET matrix in the sol-gel technique to create antimicrobial active packaging incorporating lysozyme. These films have antibacterial properties against *Micrococcus lysodeikticus* while retaining enzyme activity. [117]. The inclusion of partly purified lysozyme (187-1318 U/cm^2) into zein film changed the lysozyme release rates from 7 to 29 U/cm^2/min and enhanced high *lysozyme* doses at 4

°C. These films had an antibacterial impact on *Bacillus subtilis* and Lactobacillus plantarum. However, by including EDTA, a partially pure lysozyme-zein film could have an excellent antibacterial action against Escherichia coli. In general, this type of composite could be used to make effective food packaging materials [118, 119]. Despite the challenges of zein film production, such as the synthesis of protein aggregates because of the less-soluble nature of partially purified lysozyme, this type of active packaging is a viable candidate. This protein aggregation problem could be solved by emulsification with edible components.

To regulate the release rate of lysozyme-cellulose acetate-modified films; the structure of films could be changed from highly asymmetric and porous structures to compact ones [120, 121]. The lysozyme release rate is affected by the film surface structure; this may be due to lysozyme mechanism reasons; diffusion through the film. The diffusion mechanism of lysozyme-based films is affected by the morphological features of the films. According to the latter mentioned, asymmetric porous films lysozyme or other active ingredients-based films could be used as an innovative internal food packaging material that exhibits a slow-release behavior. More examinations are expected to explore the viability of these active food packaging films on selected foods. The covalent attachment of lysozymes to different copolymers like ethylene vinyl alcohol copolymers acts as a possible novel technique These films exhibit antibacterial activities as the same as the free enzyme on Listeria monocytogenes [122].

Lysozymes can chemically immobilize into plasma-treated polyethylene films with different concentrations [123, 124]. In either physical or chemical plasma treatment process, a polyethylene film`s surface layer contributes to immobilizing lysozyme on its surface. The results investigated that, plasma-treated /lysozyme-based film exhibits antimicrobial impact against *Micrococcus lysodeikticus*. However, in some investigations, such as the reaction of glutaraldehyde and cyclohexyl isocyanide to polyamide, lysozyme is covalently immobilized and shows more proper activity and sustains release [125].

Lactoperoxidase is another enzyme that is frequently employed as a natural antibacterial. Hypothiocyanite (OSCN-) and hypo-thiocyanous acid (HOSCN) are oxidized products of the thiocyanate ion (SCN-) oxidation, which is catalyzed by lactoperoxidase. By irreversibly oxidizing the sulfhydryl (SH) groups found in microbial enzymes and other proteins, these oxidized compounds function as antimicrobial agents by causing these biomolecules to lose their activity and ultimately die as cells. [126, 127].

4. Plant natural extracts

Natural ingredients such as plant extracts, have high levels of phenolic components, which have powerful antibacterial and antioxidant effects. Films and coatings produced from integrating plant extracts into polymers typically resulted in altered physicochemical, mechanical, barrier, antioxidant, and antibacterial properties as compared to films formed from individual components and have been employed for a wide range of purposes in polymers [128].

Plant extracts incorporated into films made of polymers will produce antibacterial packaging material [129] as in Figure (6).

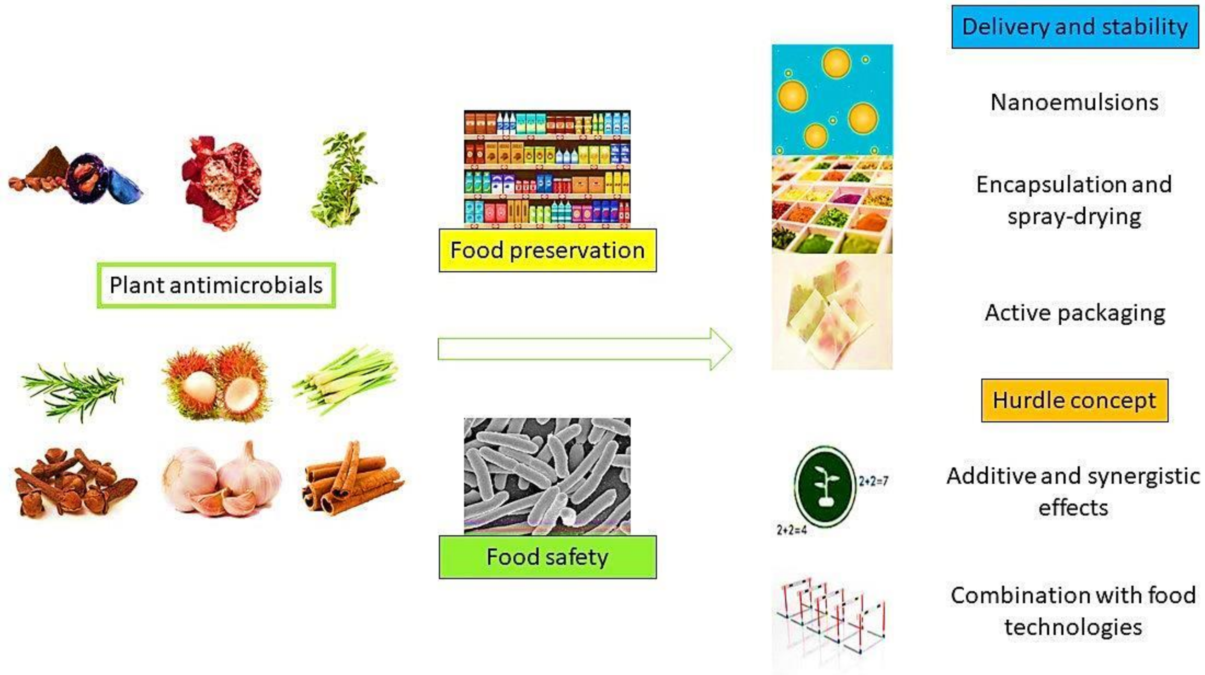

Figure (6): Antimicrobial activity of plant extract in food packaging [130].

These plant extracts inhibit or limit the growth of spoilage and pathogens of bacteria in packaged foods, thus enhancing shelf life [131]. When rosemary was added to water, it demonstrated antibacterial activity due to the presence of phenols and diterpenes such as carnosic acid, rosmarinic acid, rosmanol, isopropanol, and carnosol. nanoclay/carrageenan films [132, 133]. Whey protein films containing rosemary extracts inhibited the growth of *Staphylococcus aureus* and *Listeria monocytogenes* significantly [134]. The antimicrobial activity of κ-carrageenan films containing flesh extract and pomegranate peel has been reported [135].

The carrageenan control film has no inhibitory action against four foodborne pathogens (*Salmonella, Staphylococcus aureus, Escherichia coli, and Listeria monocytogenes*). However, both active films and their extracts demonstrated antibacterial efficacy against foodborne microorganisms. Because the pomegranate peel extraction exhibited a higher total phenol content, the films had better antibacterial action than the pomegranate flesh extract at the same extract inclusion level. Gram-negative bacteria (*E.coli and Salmonella*) were more responsive to the film composite than Gram-positive bacteria (*L.monocytogenes and S.aureus*), owing to differences in cell wall architectures between *Gram-positive* and *Gram-negative bacteria*. Edible films for packaging constructed from chitosan and Sonneratia caseolaris (L.) Engl. leaf extract was investigated [136-138]. Also, Shaimaa A. Khalid et al produced novel free-standing carboxymethyl cellulose (CMC) nanocomposite films containing nanoencapsulated flavonoids from pomegranate extract. To optimize their stability, solubility, and controlled release characteristics, the loaded flavonoids, which are recognized for their antioxidant and antibacterial activities, were nanoencapsulated in a mixture of chitosan and sodium alginate using a self-assembly method. The nanocomposite films outperformed the Pg-NPs-free CMC films in terms of suppressing bacterial growth and prolonging the shelf life of beef and poultry meat by 12 days [139].

The antibacterial activity of chitosan-based films containing natural extract was more effective against Gram-negative bacteria (*Pseudomonas aeruginosa*) than Gram-positive bacteria (*Staphylococcus aureus*), and it was attributed primarily to extract flavonoids, luteolin, and luteolin 7-o-glucoside. The inclusion of sage and rosemary extract increased the antibacterial activity of the pure chitosan film against *Escherichia coli* and *Staphylococcus aureus*. In particular, rosemary extract incorporating film outperformed sage extract with film in terms of inhibitory effectiveness against both types of bacteria [140-142].

Varghese et al. (2023) chose *Escherichia coli* and *Staphylococcus aureus* to assess the antibacterial activity of PVA/starch films when basil leaf extract was added [143]. The active films incorporating basil leaf extract showed good antibacterial efficacy against the identified infections. In general, basil extract can suppress microorganism development by a variety of mechanisms, including cytoplasmic material damage, protein coagulation, and metabolite and ion leakage.

The cruciferous plants like mustard and horseradish parts such as leaves, stem, seeds, and roots contain a flavoring ingredient known as allyl isothiocyanate (AIT) which, has a strong inhibitory activity against various spoilage food microbes. AIT has been produced chemically due to market requirements [8, 144-146]. AIT is permitted for use as a food additive [147, 148]. Because of the volatile nature of AIT, it is likely to slowly release from the package into the atmosphere around food to prevent microbial germination on food surfaces. That's why, AIT beads have been encapsulated into low-density polyethylene (LDPE) capsules [149-151]. The film thickness and temperature effects on AIT release from LDPE capsules were studied. As long as the film thickness of LDPE film decreased and temperature increased, the AIT release rate increased. The film was applied to fresh spinach; it showed the effect on the growth of molds, *E. coli*, and yeasts. The preliminary count of *E. coli* is 5.6 log CFU/leaf (colony-forming unit) decreased by 1.6–2.6 log CFU/leaf within 5 days at 4 ^{o}C.

Grapefruit seed extract (GFSE) is a naturally antimicrobial agent that has a limited inhibition effect on Gram-positive and Gram-negative bacteria growth [152-154]. GFSE has a strong germicidal, antibacterial antiseptic, fungicidal, and antiviral effect. GFSE was incorporated into chitosan, polyethylene, and polycaprolactone to produce food packaging materials with promising antibacterial properties due to the antibacterial actions of the inherent abundant phenolic compounds such as gallic acid [155-157]. The mixing of GFSE with biodegradable polycaprolactone and alginic acid increases the crystallinity of films [120, 158]. The compression molding technique was used to produce a homogeneous and smooth film. The film exhibits an extraordinary antimicrobial action against *P.aeruginosa*. GFSE showed no effect on the transparency properties of chitosan film at diverse concentrations of 0.5, 1.0, and 1.5% v/v. The increasing percentages of GFSE improve the mechanical properties of chitosan-based films especially, tensile strength and elongation %. The antifungal test assessment pointed to that the chitosan-based films tend to retard fungal growth. The polyethylene film containing GFSE fabricated via solution-coating process or co-extrusion was anti-microbially tested on ground meat at cold storage temperature (3^{o}C). The composite film reveals an improved antimicrobial action against certain microorganisms such as *S.aureus*, *E.coli,* and *B.subtilis*. Na-alginate and K-carrageenan-based films of multicomponent antimicrobial agents containing nisin, lysozyme, EDTA, and GFSE were developed that work solely and/or in combined action. The findings revealed that GFSE-EDTA is an extensively appropriate inhibitory agent and has an effect on all tested microorganisms, especially Gram-negative bacteria [120].

4.1.Natural Antimicrobials from Algae and Mushrooms

Both microalgae (diatoms) and macroalgae (seaweed) produce antibacterial compounds. Marine algae derivatives are being sought after by the pharmaceutical and food industries [159-162]. There has been little investigation into the antibacterial effects of algae for food preservation purposes. Several researchers have investigated the antibacterial properties of algae. This type of chemical substance was discovered by screening macro- and microalgae [163]. Synechocystis spp and Himanthalia elongata extracts were found to have antioxidant properties as well as antibacterial activity against *S. aureus* and *E.coli* [164] When they investigated extracts from Haligra spp. that were efficacious against S. aureus. Antimicrobial activity of Hymanthalia elongata, Saccharina latissima, Laminaria digitate, Padina, and Dictyota against *Enterococcus faecalis*, *Salmonella*, *B. cereus*, *L. monocytogenes*, *P. aeruginosa* and *E. coli* has been reported [165, 166].

Carrageenan and alginates, two algae-derived chemicals, are beneficial for food applications and films; these compounds, when combined with other natural antimicrobials, will expand their applicability [167]. Alginates and carrageenan have been employed in a variety of ways in the food sector, including: producing nanocomposite films loaded with essential oils, increasing its efficacy against *L. monocytogenes* [168, 169], In combination with chitosan and isothiocyanate in a food packaging film and active against *C. jejuni* [170], When coupled with EDTA in a film, perhaps reducing Salmonella populations [171].

Antimicrobial nanocomposite films are being developed to inhibit the growth of foodborne bacteria. First, they investigated the antibacterial properties of cinnamon, caraway, cumin, clove, coriander, and marjoram essential oils against *E. coli*, *S. aureus*, and *L. monocytogenes*. The essential oils of marjoram, cinnamon, and clove, which are the most effective against bacteria, were then mixed into nanocomposite films composed of alginate or clay and tested for 12 days. In all matrices, marjoram (1.5%) had the strongest effectiveness against microbes, with populations of the three bacteria dropping by 6.3, 4.5, and 5.8 logs, respectively [172].

Four Campylobacter jejuni strains were evaluated on vacuum-packaged chicken breasts at 4 ^{o}C with allyl isothiocyanate enclosed in an edible coating (containing 2% chitosan, and 0.2% carrageenan) [173]; After 5 days, coatings with 50 or 100 L/g allyl isothiocyanate reduce the number of microorganism cells to levels under the detection limit. The previously described carrageenan/chitosan coating was examined for its capacity to prevent Salmonella population on fresh chicken breasts; the edible coating included allyl isothiocyanate, mustard, EDTA, or their combinations [174]. Salmonella counts were reduced within 2.3 log CFU/g at 4 _C after 21 days in coatings containing 250 mg/g of mustard or 50 L/g of allyl isothiocyanate mustard seeds. Mushrooms, like other fungi, have antibacterial and antioxidant properties [175].

In vitro, wild Laetiporus sulphureus (Bull.) Murrill fruiting body extracts showed antibacterial action against bacteria such *as E. coli, Candida albicans, S. aureus, Candida parapsilopsis, S. epidermidis* and *Enterococcus faecalis*. Aphyllophorales edible mushroom extracts also demonstrated antimicrobial action [176], Agaricus [177], Armillaria mellea, Meripilus giganteus, Morchella costata and M. elata, M. esculenta var. vulgaris, M. hortensis, M. rotunda, Paxillus involutus, and Pleurotus eryngii and P. ostreatus [178]. Methanolic extracts of six edible wild mushrooms (Lycoperdon perlatum, Ramaria formosa Cantharellus cibarius, Clavaria vermiculata, Pleurotus pulmonarius, and Marasmius oreades) were employed [179].

All of the isolates had a high content of phenols and flavonoids with antimicrobial activity against several pathogenic bacteria *(E. coli, S. aureus B. subtilis, and P. aeruginosa*) and fungi (*Candida albicans*), showing that the concentrations of the components directly impact the capability of the isolated mushrooms against the microorganisms. The fatty acids from Agaricus essettei, *A.bisporus*, and *A. bitorquis* extracts discovered that linoleic and palmitic acids were dominant and efficacious against Gram-positive bacteria (*Micrococcus luteus, Bacillus cereus*, *Bacillus subtilis,* and *Micrococcus flavus*) [180]. The antimicrobial activities of the mushrooms mentioned above against 11 microorganisms *(E. cloacae, Bacillus subtilis, E. coli, Enterobacter aerogenes, Enterococcus faecalis, Proteus vulgaris, S. typhimurium, Sarcina lutea, S. aureus, Bacillus cereus, and the yeast Candida albicans*). The species having the highest activity against bacteria and yeast were *P.ostreatus* and *M.giganteus* [181].

Conclusions and Future Trends

Food packaging systems are constantly influenced by rising demand for minimally processed goods, shifting eating patterns, and food safety concerns. Minimally processed foods promote the proliferation of dangerous microorganisms, jeopardizing quality and safety. As a result, the necessity for longer food shelf life and protection against foodborne infections, combined with customer demand for minimally processed foods with no or few synthetic ingredients, drives the development of novel technologies such as antimicrobial packaging. It is a type of active packaging that can release antimicrobial chemicals to inhibit the activity of specific microbes, increasing food quality and safety during long-term storage. However, antimicrobial packaging continues to be a difficult technology.

The development of active antimicrobial packaging is currently moving away from the use of oil-derived, nonbiodegradable, noncompostable plastic materials with synthetic antimicrobial agents like organic acids and antibiotics to the use of biodegradable, sustainable, and environmentally friendly packaging materials like zein, chitosan, cellulose or, starch with natural antimicrobial compounds like bacteriocins, bacteriophages, and essential oils, among others.

The integration of these new products into these innovative packaging materials presented new challenges due to the incompatibility of antimicrobial packaging materials and, above all, the reduced stability of these natural-based compounds, which are more exposed to heat and light-induced degradation. Additionally, certain antimicrobials, like essential oils and their main constituents, have inherent volatilization, which means that adding them to packaging materials carries additional risks. To address these issues and improve the durability and efficacy of these new natural-based antimicrobial packaging, the food industry and food scientists have begun to search for novel approaches.

It is important to find new natural food preservatives because consumers are demanding food free of artificial preservatives. These novel strategies help to reduce foodborne pathogens and increase food shelf life. Finding natural antimicrobials must be economical from an economic perspective. Combining various natural antimicrobials with food preservation methods is one possible alternative. Natural antimicrobial compounds may bind with certain food components, limiting their effectiveness, because food matrices are complex. Therefore, more investigation is required to find the most effective antimicrobial delivery method as well as the ideal concentrations of these naturally occurring antimicrobial compounds.

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