

## PVC in food packaging: challenges and prospects of date seeds as natural antimicrobial agents – a review

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### Abstract

Polyvinyl chloride (PVC) is the most widely used material in food packaging production. It is approved for use in food contact applications worldwide, and numerous PVC/additive mixtures are already listed on European incomplete additive lists, such as those described in EC Directive 2002/72 and its following amendments. However, the use of PVC in contact-sensitive applications, such as food packaging, as advised by the Zero Waste Europe office, raises serious concerns. However, phasing out PVC in packaging limits consumer choice without providing environmental benefits. Currently, common natural antimicrobials found in food packaging include lysozymes, organic acids, bacteriocins, essential oils, chitosan, grapefruit seed extract, allyl isothiocyanate, and polysaccharides. The production of natural antimicrobial films involves either direct coating or the incorporation of synthetic or natural antimicrobial agents into the film. Although many natural antimicrobial agents have received a generally recognized as safe (GRAS) designation, cost and scalability continue to be significant challenges to expanding their use. In price-sensitive markets, natural antimicrobials are less practical due to their high production costs. Date seeds are considered potential prototypes for developing novel, affordable, and safe antimicrobial films. They include bioactive substances with antiviral, antibacterial, and antioxidant qualities. Additionally, date seeds are a source of oil rich in phenols, tocopherols, and phytosterols. This review discusses the nature, biocompatibility, and properties of the most common antimicrobial agents in enriched PVC films. It also emphasizes how date seeds can be used as organic microbiological materials to preserve food during packing. Since date seeds are a low-value byproduct, their disposal is costly, making their utilization economically advantageous.

**Keywords:** Antimicrobial; Bacteriocins; Date seeds; PVC; Packaging

### 1. Introduction

The main cause of food spoilage and decay during post-processing, transportation, and storage is the growth of foodborne illnesses and other well-known bacteria on food surfaces. One of the most promising active packaging technologies involves the production of antimicrobial films by incorporating natural or synthetic antimicrobial agents into

polymeric films [1, 2]. Synthetic thermoplastic polymers are the most popular packaging materials due to their numerous benefits, including high strength, heat sealability, transparency, and transfer resistance. Plastics are generally affordable, offer good barriers to heat and oxygen, and resist tensile and tensile stresses [3]. Despite these advantages, increasing environmental concerns have prompted a review of traditional packaging polymers. The goals of traditional food

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packaging are to keep food products safe, preserve quality, and increase shelf life. Products derived from natural sources are essential to current strategies for meeting consumer needs and maintaining food quality throughout time. Most packaging materials are petroleum-based from nonrenewable resources, and synthetic plastics are not biodegradable. Since packaging accounts for 30% to 40% of municipal solid waste, the amount of solid waste generated poses an environmental challenge that needs immediate attention. Many studies are ongoing to address these issues by exploring ways to valorize and reuse waste without harming the ecosystem. Various substances, including metal ions, alcohols, ammonium compounds, amines, organic and inorganic acids, and metals such as copper and silver, have been researched [4, 5]. A suitable rate of controlled release of antimicrobial agents during food preservation is also made possible by packaging materials [6]. Natural substances such as antimicrobials and antioxidants are effective in laboratory settings. When incorporated into food packaging films, antimicrobial agents often prevent the growth of unwanted microorganisms [7-9]. Natural antimicrobial agents are becoming more popular because they are seen as safe for use in the food industry. These include the possibility of using antimicrobial enzymes such as lysozyme, lactoperoxidase, chitinase, and glucose oxidase, as well as bacteriocins such as nisin, pediocin, and lactacin, as biopreservatives [10, 11]. Ensuring food safety involves linking active antimicrobial coatings with the diffusion behavior of the agents on food surfaces, where microbiological degradation begins. In other words, the diffusion rate must align with the product's concentration and shelf life, both of which must remain within safe limits. Additionally, antimicrobial agents should be released gradually during storage [12]. Importantly, research on how antimicrobials are released from packaging films has focused on the diffusion rate [13, 14] and biodegradability [15-18], which influence antimicrobial diffusion within the polymer matrix. Numerous studies have investigated the mechanisms of antimicrobial release from plastic films [19-23], the diffusion of antimicrobials from packaging materials [24-26], and extensively covered topics such as migration testing, nanomaterial safety, industrial scaling, and regulatory updates [27-32]. Because of their inherent film qualities, such as flexibility, transparency, and ease of processing, plastics are

widely used in food packaging and antimicrobial food packaging, and their use continues to grow. These qualities, flexibility, transparency, and ease of processing, have led to a sharp increase in plastic use for food packaging and antimicrobial food packaging. These films are available in oriented and unoriented types and are typically made by blending PVC resin, plasticizers, and other additives. PVC is permitted for use in food contact applications worldwide. According to EC Directive 2002/72 and its updates, numerous compounds routinely utilized in PVC are already on European additive lists. Like other thermoplastics, PVC packaging can be mechanically recycled at the end of its life, and recycling facilities for bottles and trays are established across Europe. PVC has also been combined with algae extracts to create bioplastic films, which are potentially environmentally friendly alternatives to traditional plastics [33]. Extracts from *C. reinhardtii* demonstrate antimicrobial activity against various bacteria and fungi, suggesting that they could be useful in antimicrobial packaging [26]. Therefore, removing PVC from packaging options would limit consumer choice without providing additional environmental benefits. The primary objective of this research was to explore the potential integration of various natural antibacterial agents suitable for the food industry into the PVC matrix to produce effective bioplastic films.

## 2. Common natural antimicrobial agents

In modern medicine, natural substances, especially secondary metabolites, are a significant source of pharmaceuticals. Antimicrobials can originate from microorganisms, plants, or animals. Animal-based natural antimicrobial agents include chelators such as chitosan, lysozyme, and lactoferrin [34, 35]. Essential oils derived from citrus fruit peels, seeds, bulbs, or pods of various plants are known as plant-based antimicrobial agents [36, 37]. Similarly, microbes produce inhibitory substances that antagonize nearby pathogens [38]. Table 1 [39-41] presents classification of natural antimicrobial agents, targeted bacteria, processes, food applications, and limitations. Additional details on microbial, plant, and animal origins, types, and uses can be found elsewhere [42-47]. A few natural antimicrobials used in food packaging are briefly discussed in the following sections.

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**Table 1. Classification of natural antimicrobial agents according to their sources, targeted microbes, mechanisms, food applications, and limitations.**

Microbial sources	Animal sources	Plant sources	Targeted Microbes (Applications)	Mechanisms	Limitations
Bacteriocins	Proteins	Essential Oils	<i>Listeria</i> , <i>C. perfringens</i> , <i>S. aureus</i> (Cheese, yogurt, cured meats)	Layered packaging	Strong aroma /flavor
Nisin, Pediocin, etc.	Lactoperoxidase,	Plant extracts	(+) Gram bacteria <i>Listeria</i> , <i>S. aureus</i> (Cheese, milk, seafood coatings)	Inflammation of cells	Limited spectrum against (-) positive bacteria
Pediocin	Lactoferrin	Lamiaceae families	<i>S. aureus</i> , <i>E. coli</i> , <i>P. aeruginosa</i> (Strawberries, raspberries, beef)	Disrupt cell membrane	allergenicity and solubility issues
Bacterial Cell Products	Lysozyme	Fabaceae families	Mesophilic bacteria (Sprouts, cabbage)	DNA damage	pH sensitive
Organic acids	Polysaccharide	Asteraceae families	<i>S. aureus</i> , Salmonella (Dairy products)	Mitochondria damage	Lipid interactions reduce the antimicrobial efficacy
Carbon dioxide	Chitosan	Cinnamomum verum	<i>Pseudomonas</i> , <i>Aeromonas</i> (Fish fillets)	Protein denaturation	Impact on sensory properties
Hydrogen Peroxide	Enzymes	<i>Rosmarinus vulgaris</i>	spoilage organisms (Cheese, dairy products)	Electrostatic disruption	Shelf-life stability
Reuterin	Lactoperoxidase, Lysozyme	<i>Thymus vulgaris</i>	Molds and yeasts (Bakery products)	Lysosomes' function disturbance	Cost and scalability

## 2.1. Essential oils

Aromatic oil liquids containing up to sixty phenolic components are called essential oils. They are mainly produced synthetically or extracted from plants. Besides their use as flavorings, fragrances, and medicines, they also have antiviral, antibacterial, and insecticidal properties, among other biological functions [35]. Data on the composition of different essential oils have been published in several sources. Depending on the harvest time and location, different plant species can have varying essential oil compositions [36-38, 47]. Linalool, E-2-decanal, trans-cinnamaldehyde, carvacrol, thymol,  $\gamma$ -terpinene, p-cymene,  $\alpha$ -pinene,  $\alpha$ -tujone, bornyl acetate, camphor, 1,8-cineole,  $\gamma$ -pinene,  $\alpha$ -tujone,  $\gamma$ -eugenol, and eugenyl acetate are major components of antimicrobial essential oils. Figure 1 displays the structural formulas of some components with strong antibacterial activity. Hydrophobicity is a key property of essential oil components, enabling their incorporation into bacterial cell membrane lipids and mitochondria, disrupting their structure and increasing their permeability [48, 49]. Many essential volatile plant oils are known for their wide antimicrobial range; phenolic compounds are generally the most effective. Numerous reports have shown that essential oils are more effective at inhibiting gram-positive bacteria than gram-negative bacteria. Examples

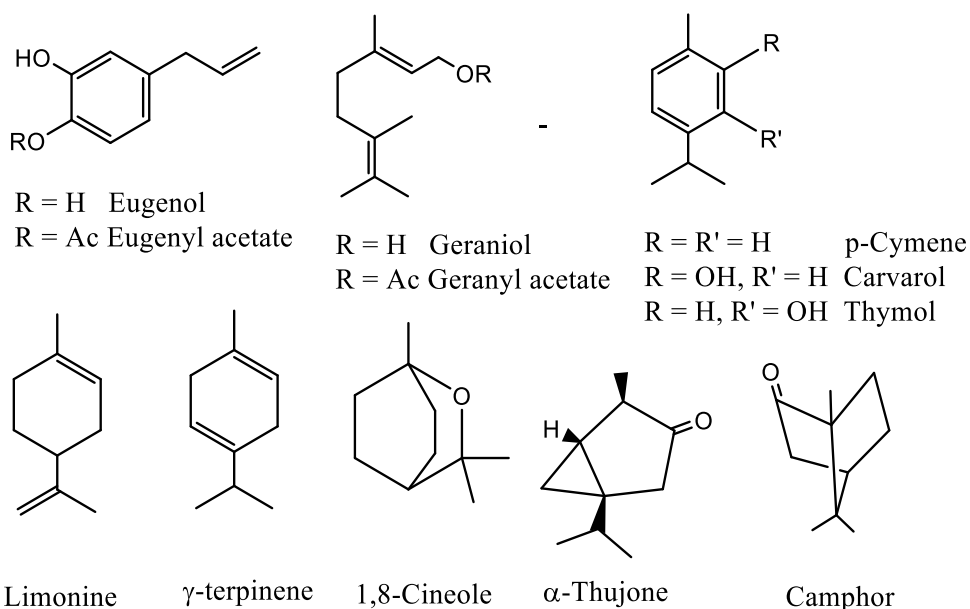
of the former include clove, oregano, sage, vanillin, rosemary, and thyme, whereas examples of the latter include oregano, cinnamon, citral, and garlic oil [50]. Today, essential oils are most commonly used in food applications, including flavorings, fragrances, medications, dental root canal sealants, antiseptics, and feed additives for weaned piglets and lactating sows [51-54]. The potent effects of these oils are mainly due to higher concentrations of secondary metabolites, such as aldehydes, ketones, and phenolic compounds, which make them highly lipophilic and volatile. Importantly, although many essential oil components are approved for various uses, some research indicates potential toxicity and irritation. For example, during root canal therapy, eugenol, menthol, and thymol have been reported to irritate oral tissues. Regular exposure to certain constituents may cause allergic contact dermatitis. Several oils used in aromatherapy, paramedicine, and medicine have demonstrated spasmolytic or spasmogenic effects. Therefore, more safety studies are necessary before widespread or high-concentration essential oils can be used in food. Additionally, direct addition to food products is limited by their high volatility, hydrophobicity, tendency to lose flavor, and susceptibility to oxidation and photooxidation. Synergistic effects with other preservation strategies are thought to be advantageous for reducing the negative effects of large concentrations of these substances [42].

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## 2.2. Bacteriocins

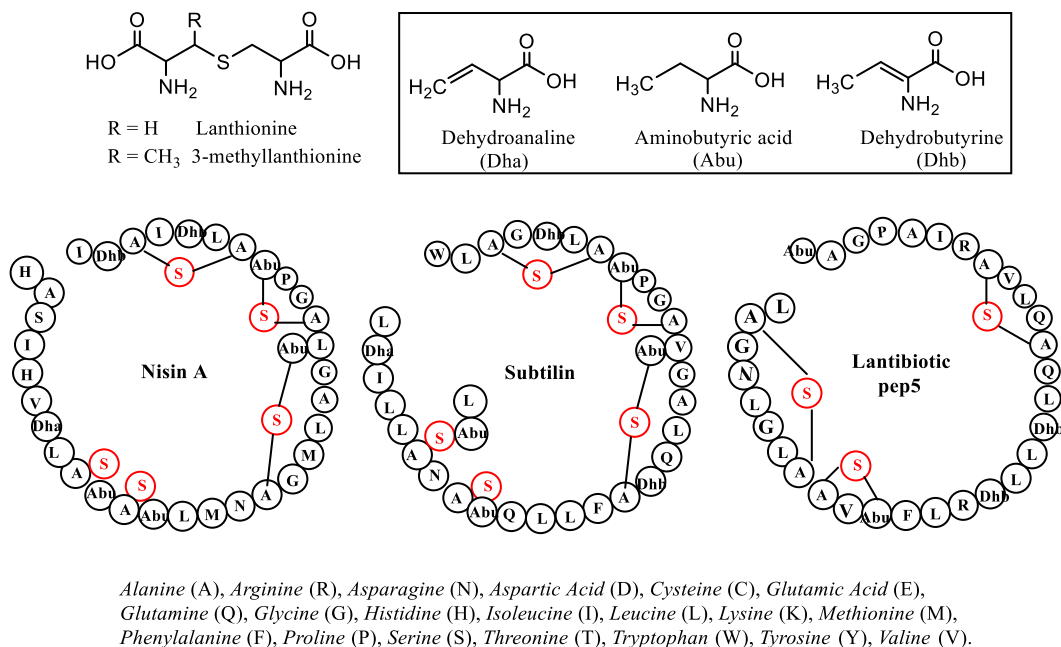
substances are produced by bacteria, encoded by genes, and synthesized by ribosomes. They have been explored as promising natural sources of antimicrobial agents because of their ability to antagonize other bacteria, especially closely related species [55-59]. These natural peptides are produced by bacteria living in competitive polymicrobial environments and are of great interest as potential antimicrobial agents against different bacterial, fungal, and viral species [60], including resistant structures such as bacterial biofilms [61, 62]. The diversity of bacteriocins among bacteria provides a broad spectrum of activity [63-65]. Owing to this high diversity of bacteria, a wide variety of bacteriocins have been identified, with some bacteria capable of producing several types [66]. This broad range of antimicrobial molecules enables numerous biotechnological, industrial, and pharmaceutical applications [67]. Currently, bacteriocins are used in two main sectors: combating antibiotic-resistant bacteria and the agrifood industry. In the first sector, bacteriocins were seen as weapons bacteria use to survive, and their potential to fight drug resistance has gained increasing interest [68, 69]. In the second, however, they are rapidly broken down by proteolytic enzymes and are considered safe for human use [54].

Based on their size, shape, chemical composition, or mode of alteration, bacteriocins can be categorized into multiple types [70]. Gram-positive bacteria produce four different classes of bacteriocins [71]: small-sized (<5 kDa) lantibiotics, which contain unusual amino acids such as dehydrated amino acids, lanthionine, and 3-methylanthionine, which form multiple ring structures [72, 73]; nonantibiotics, which do not contain unusual amino acids [74, 75]; members, which exhibit a linear structure with bisulfide bridges (antilisterial bacteriocins) [76, 77]; antibiotic action, which requires the production of two-peptide bacteriocins ( $\alpha/\beta$ ), including plantaricin NC8, lactococcin G, and lactococcin Q [78]; and small bacteriocins associated with a leader peptide sequence, including one or two cysteine residues, such as cystibiotics, thiolbiotics, and other molecules such as lactococcin A, divergicin A, or acidocin B. Finally, all the bacteriocin classes combine. The third class of gram-positive bacteriocins includes large peptides (>30 kDa), such as zoocin A, lysostaphin, and helveticin J and V, which exhibit antibacterial activity via enzymatic mechanisms that disrupt the bacterial cell wall [79, 80]. The final type of gram-positive bacteriocin disrupts bacterial cell membranes and is characterized by its lipid- or carbohydrates, such as plantaricin S or leuconocin [81].

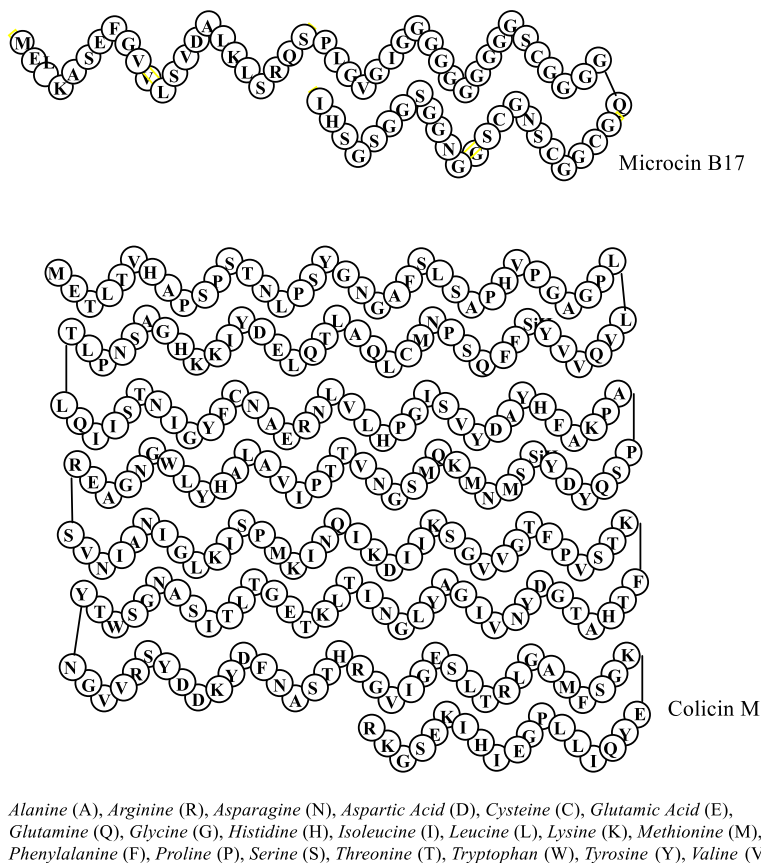


**Figure 1.** Chemical formulas of some essential oils containing components possessing high antibacterial properties.

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**Figure 2.** Examples of lanthionine-containing antibiotics found in bacteriocins made by Gram-positive bacteria (modified from Garnizova *et al*) [88].



**Figure 3.** Some antibiotic classes retrieved from bacteriocins produced by *gram-negative bacteria* (*E. coli* source) (modified from Wu *et al*) [89].



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On the other hand, gram-negative bacteria produce bacteriocins, which are important antimicrobial peptides; most of these peptides are isolated from *Escherichia coli* strains, whereas other genera, such as *Pseudomonas* or *Klebsiella*, also produce antimicrobial peptides [82]. This type of bactericide can be divided into four categories: colicins, which are bacteriocins with molecular weights greater than 10 kDa [83, 84]; colicin-like bacteriocins, which are produced by bacteria such as *Klebsiella* spp., klebicins, *P. aeruginosa*, and S-pyocins [85]; microcins, which are small peptides (<10 kDa) [86]; and phage tail-like bacteriocins [87, 88], which include the production of a needle-shaped protein structure, genes involved in peptide release, and regulatory genes. The bacteriocins in this group are R- and F-pyocins produced by *P. aeruginosa* that stopped membrane potential, leading to pore formation in the bacterial membrane. The chemical structures of certain bacteriocin components produced by gram-positive and gram-negative bacteria are displayed in Figure 2 and Figure 3, respectively, along with their amino acid sequences [89, 90].

### 2.3. Lysozyme

Lysozyme, N-acetylmuramic hydrolase, is a tiny, monomeric protein (Figure 4) that has nine negatively charged amino acid residues, namely, asparagine and glutamine (7 Asp, 2 Glu), and its eight cysteine residues are joined by four disulfide linkages and positively charged amino acid residues, namely, lysine and arginine (6 Lys, 11 Arg). The  $\beta$ -(1, 4)-glycosidic link that connects N-acetyl glucosamine and N-acetylmuramic acid in bacterial cell wall peptidoglycans can be broken down by lysozyme. Consequently, its antimicrobial effectiveness against gram-positive bacteria has been demonstrated in numerous reports. [91] Several varieties of lysozymes are known, such as phage, bacterial, and plant versions; however, based on structural differences, three main families are recognized: c-type, g-type, and i-type. With 129 amino acids (14.3 kDa). The primary structure of c-type lysozyme includes four unbroken disulfide bonds (6 C–127 C, 30 C–115 C, 64 C–80 C, and 76 C–94 C) formed by cysteine residues, six tryptophan (W) residues (W–62 and W–108), three tyrosine (T) residues, and three phenylalanine (F) residues [92, 93]. Lysozymes are commonly used by pharmaceutical companies to treat bacterial, viral, and inflammatory diseases. The peptidoglycan  $\beta$ -1,4-glycosidic

bond is hydrolyzed, and muramidase activity degrades the murein layer, decreasing the mechanical strength of the bacterial cell wall and ultimately leading to bacterial mortality [94]. The bactericidal action of lysozyme is mostly restricted to certain Gram-positive bacteria because the outer surface of Gram-negative bacteria usually has a protective coating of lipopolysaccharide (LPS) as well as proteins and phospholipids that prevent lysozyme from accessing the peptidoglycan layer [95]. Notably, lysozyme's ability to bind to food additives, including food colors and antioxidants, provides deeper insight into toxicity profiles and reveals metabolic pathways of food ingredients [96]. Antimicrobial enzyme immobilization in packaging is a promising approach in active food packaging. This method can preserve the activity of antimicrobials by preventing direct contact with food components such as lipids and proteins. In contrast to when the same quantity of lysozyme is merely dispersed or sprayed on the food surface, immobilizing lysozyme in films isolated from whey protein guarantees that it maintains a minimum inhibitory concentration for a longer period at the film's outer surface and/or the film–salmon interface. In hydrophilic whey protein isolate (WPI) films, lysozyme and polyacrylic acid can form a combination that enables gradual, sustained release, long-term antibacterial activity, and substantial food-preservation potential [97]. The effectiveness of lysozyme-based antimicrobial packaging in food preservation stems from its unique properties, particularly its strong bacteriostatic activity against Gram-positive bacteria.

### 2.4. Organic acids

Organic acids are naturally occurring substances present in various foods and are largely produced by microbes. They are widely used as antimicrobial agents in the food industry and have broad-spectrum antibacterial action [98, 99]. A comprehensive review of the mechanisms of preservatives and antiseptics revealed that organic acids are more effective than mineral acids as antimicrobial agents [100]. Organic acids constitute the third-largest global market for production and are frequently utilized as antimicrobial agents in the food sector [101]. Short-chain organic acids are often associated with antibacterial activity because they have 10 or fewer carbon atoms. Furthermore, several important characteristics affect the effectiveness of organic acids, including their ionic form, pKa value, molecular weight, minimum inhibitory

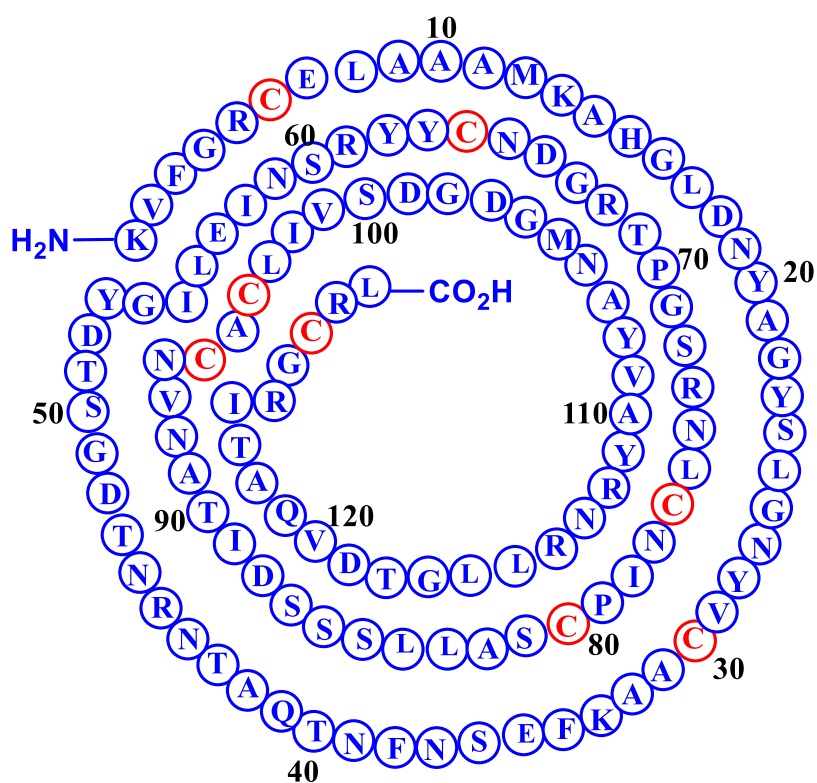
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concentration, the microbes they target, the food matrix buffering capacity, and the length of time they are exposed to the acid [102]. With pKa values of 3 to 5, most organic acids with antibacterial properties are suitable for use in food preservation [103]. Because of their low molecular weight and straightforward molecular structure, which enable effective penetration into microbial cells, they have significant advantages when used as preservatives. After entering, they disrupt intracellular functions, which eventually results in cell death [104]. The food industry often uses approved organic acids, whose structural formulas and pKa values are shown in Figure 5. According to recent research, combining organic acids with bioactive substances, such as plant extracts and essential oils, increases antimicrobial activity, enhancing food safety and quality. By preventing oxidation, enzymatic breakdown, and microbial growth, this synergistic effect helps maintain sensory qualities and nutritional value [105]. As food preservatives, oil stabilizers, antimicrobials, active ingredients in food packaging, and stabilizers during food processing, organic acids serve a variety of purposes [106, 107]. For example, tartaric acid derivatives used as antimicrobial agents in packaging have demonstrated effectiveness against *S. maltophilia*, *P. syringae*, *P. aeruginosa*, and *X. beticola*, with inhibition observed within hours at 37°C. It has also been shown that tartaric acid possesses antifungal properties against *Aspergillus fumigatus*, *Candida albicans*, *Malassezia furfur*, and *Trichophyton mentagrophytes* var. *mentagrophytes* [108].

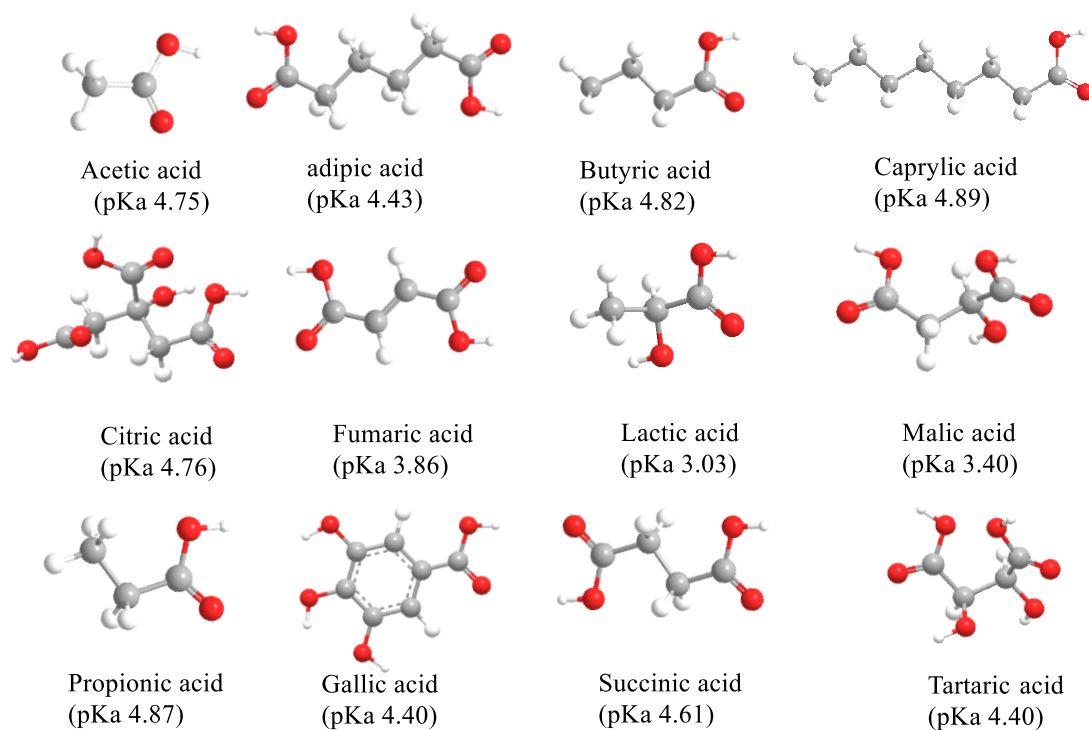
### 2.5. Chitosan

Chitin ( $\beta$ -(1 $\rightarrow$ 4)-poly-N-acetyl-D-glucosamine) is one of the most abundant natural polysaccharides found in insects, lobsters, shrimp, and crabs. It is the main source of chitosan (Figure 6), which is obtained by deacetylating the N-acetyl (N-OAc) group of chitins [109, 110]. The antibacterial and antifungal properties of chitosan have been extensively studied and documented. Its antimicrobial properties are affected by several factors, such as the origin, pH, molecular weight, and degree of de-N-acetylation. Chitosan is soluble only in diluted organic acids such as 1% acetic acid and formic acid at pH values below 6.0. Around its pKa value, which is between pH 6 and 6.5, the solubility-insolubility transition takes place. Below pH 6.0, the  $\text{NH}_3^+\text{-C}_2$  group of chitosan becomes

protonated, making it a water-soluble cationic polyelectrolyte. Conversely, at pH values above 6.0, the  $\text{NH}_2\text{-C}_2$  group behaves as a basic group, rendering the polymer insoluble. Therefore, the solubility of chitosan primarily depends on the degree of de-N-deacetylation along its main chain [111]. The antimicrobial effects of chitosan can be extracellular, intracellular, or both, depending on the target site. Its molecular weight determines whether it can penetrate cell surfaces to exert intracellular antimicrobial activity. Shorter-molecular-weight chitosan can act both extracellularly and intracellularly, impacting vital biological processes [112, 113]. Higher-molecular-weight chitosan, however, cannot typically penetrate the cell wall or membrane; its antimicrobial activity mainly involves altering cell permeability and blocking nutrient and metal uptake externally [114]. Some studies suggest that low-molecular-weight chitosan has stronger activity against gram-negative bacteria, whereas high-molecular-weight chitosan has a greater effect on gram-positive bacteria [115]. This difference is attributed to differences in cell wall structure: Gram-positive bacteria have thicker peptidoglycan layers, whereas Gram-negative bacteria contain more lipopolysaccharides [116]. Chitosan also has notable fungicidal effects on various fungal pathogens that affect plants and humans. The minimum inhibitory concentrations vary depending on factors such as those mentioned above, as well as the specific fungus targeted [117]. Owing to its excellent biocompatibility and low toxicity, Chitosan has attracted significant interest as an excipient in pharmaceutical and biomedical applications, particularly in nanoparticle or gel formulations, owing to its cationic nature. The growing demand for natural preservative alternatives has driven research into chitosan-based products with antimicrobial properties. With antifungal, antibacterial, and antiviral properties that have drawn interest from the food sector, it is regarded as a safe biopolymer that can be used orally. Among the many applications of chitosan in agriculture are soil enrichment, foliar spraying, seed coating, hydroponic supplementation, and addition to plant tissue culture media. Among these methods, seed coating and foliar spraying are especially beneficial. Chitosan is a valuable component to produce edible antibacterial films due to its unique properties [118].



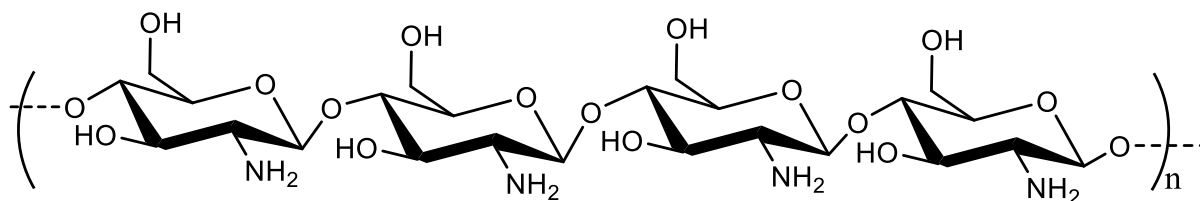
**Figure 4.** Structure of lysozyme (modified from *Wu et al*) [89].



**Figure 5.** The permitted organic acids used in the food industry, their structural formula and pKa values (modified from *Sorathiya et al*) [98].



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**Figure 6.** Chitosan [poly-(b-1 → 4)-2-amino-2-deoxy-D-glucopyranose)].

As a nontoxic, antibacterial biopolymer, chitosan has shown promise as a useful matrix for edible films that contain essential oils [119, 120].

## 2.6. Allyl isothiocyanate

Allyl isothiocyanate (AITC) results from the enzymatic hydrolysis of sinigrin, as shown in Figure 7. It is one of many isothiocyanate derivatives found in plants and is produced when the enzyme myrosinase breaks down glucosinolates, which are sulfur-containing secondary metabolites that are present only in the plant order Brassicales. Myrosinase (EC 3.2.3.147), the only glucosylhydrolase known to be able to break the C (1)-S bond of glucosinolates, typically coexists with glucosinolates in nature [121, 122]. Glucosinolates are divided into different groups based on the alkyl group attached to the isothiocyanate functional group, with sinigrin (2-propenyl glucosinolate) serving as the precursor for AITC [123, 124]. AITC exhibits potent antimicrobial activity against human pathogens in both liquid media and vapor form, particularly against bacteria with multidrug-resistant phenotypes, for which new therapeutic options are urgently needed. For example, its antimicrobial effects have been documented against such organisms as *P. aeruginosa*, *S. aureus*, *E. coli* CECT 434, *E. coli* O157:H7, and *C. jejuni* [125]. Inhibiting thioredoxin reductase and acetate kinase, interfering with the sulfhydryl groups of enzymes, and damaging the integrity of the *E. coli* cell membrane are the mechanisms of action. Notably, the ability of AITC to inhibit bacteria at all growth stages and its strong vapor-phase activity supports its potential use in food preservation [126].

## 2.7. Lactoferrin

Lactoferrin is a member of the nonheme iron-binding protein family. Its amino acid sequence shows 51% high similarity across different species. The N- and C-lobes are joined by an  $\alpha$ -helix in a polypeptide chain [127], and each

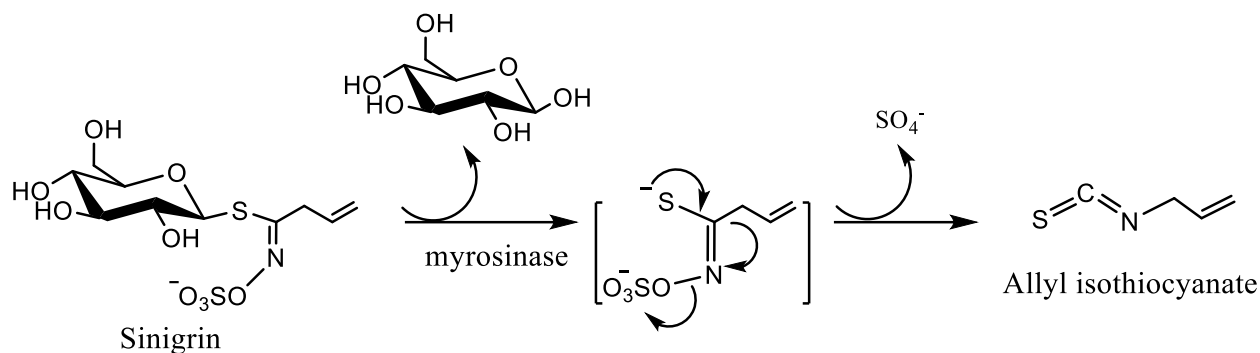
lobe has a high affinity for binding a single ferric ion. Because this binding is reversible, lactoferrin can exist in either an iron-free or iron-bound state. When iron binds, a conformational change occurs, creating a ‘closed’ protein that is more resistant to proteolysis than the open, more flexible, iron-free form. Among the primary proteins found in all exocrine secretions, such as colostrum, milk, tears, saliva, seminal and gastrointestinal fluids, nasal and bronchial mucosa, and plasma, lactoferrin is a naturally occurring substance in humans. The newborn gut microbiota is initiated, developed, and in part shaped by breast milk, the main source of lactoferrin in the infant gut. Several short peptides are produced and characterized by proteolytic enzymes [128]. Upon enzymatic hydrolysis of the amino acid sequences of oligopeptides from different animals, several significant features, such as the highly cationic nature of the peptides and the retention of hydrophobic residues, such as valine and tryptophan, are observed across species. The lactoferrin amino acid sequences of the three mammalian species are shown in Figure 8.

Lactoferrin has antibacterial, antiviral, antifungal, immunoregulatory, and anti-inflammatory properties. Other studies emphasize its role in promoting fibroblast and keratinocyte proliferation and migration, which are essential for wound healing because they modulate inflammatory responses and regulate the activity of these cells. Additionally, it supports the granulation phase by balancing fibroblast functions, including hyaluronic acid synthesis and collagen breakdown [129].

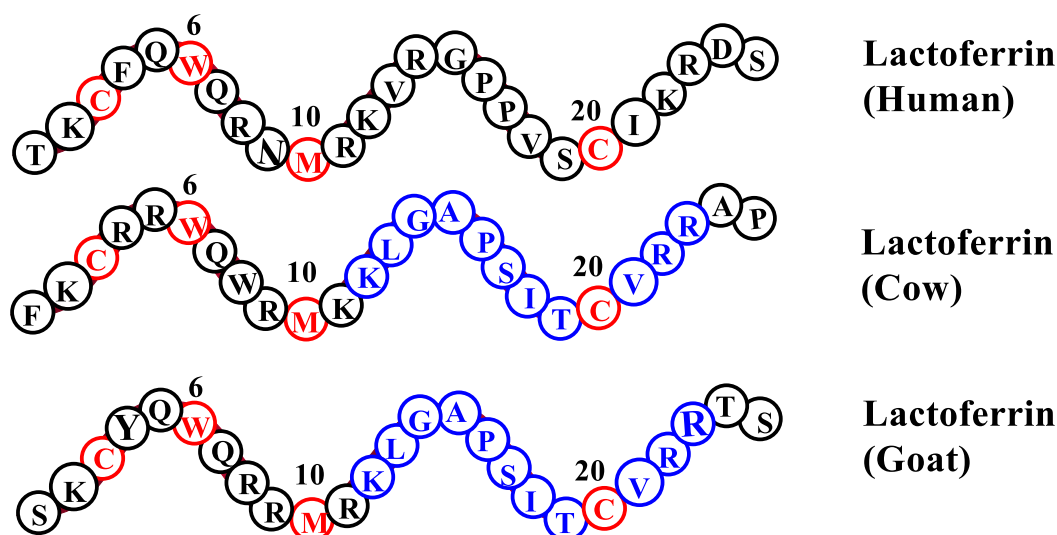
## 2.8. Grapefruit seed extract

Grapefruit seed extract is a commercial product made from the seeds and pulp of grapefruit (*Citrus paradisi* Macf.), a subtropical fruit tree in the Rutaceae family.

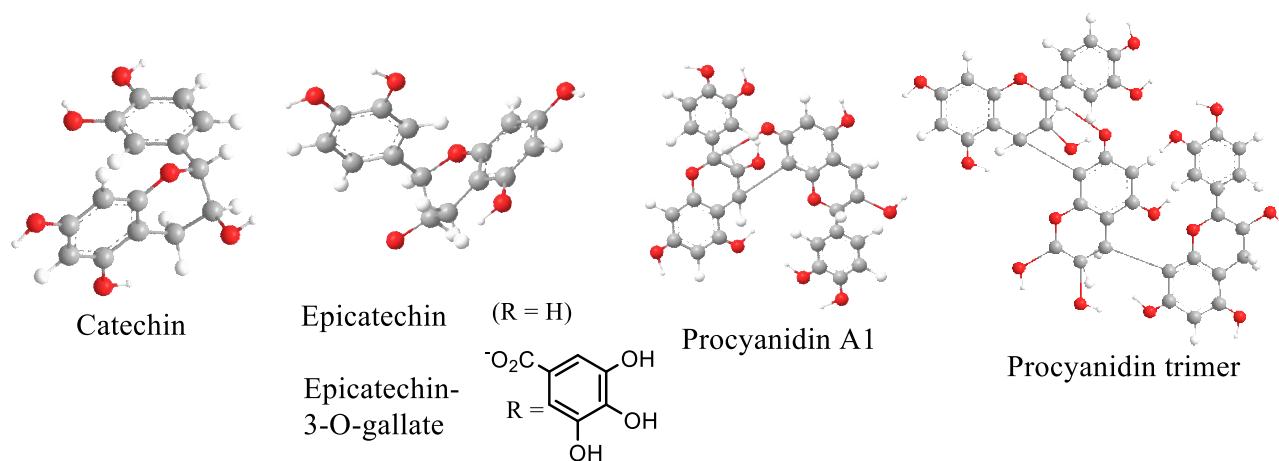
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**Figure 7.** Proposed mechanism of allyl isothiocyanate biosynthesis via Myrosinase-catalyzed sinigrin hydrolysis.



**Figure 8.** Lactoferrin amino acid sequences in humans, cows, and goats (where tryptophan (W), methionine (M), and cysteine (C) are held steady) (modified from *Bruni et al*) [129].



**Figure 9.** 3D Structure of some phenolic compounds found in grapefruit seed extract.

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Grape seed (*V. vinifera* L.) extraction via a 20 % ethanolic solution and/or hot water yields large amounts of polyphenolic compounds (Figure 9), such as procyanidins, including dimeric, trimeric, and tetrameric forms; epicatechin; epicatechin-3-O-gallate; flavanols; and catechins. It also contains small amounts of fat, fiber, sugars, organic acids such as citric and malic acids, and protein [130-132].

The antibacterial properties of extracts from grapefruit seeds have been documented in many studies, and their effectiveness is linked to flavonoids such as naringin, quercetin, kaempferol, tocopherols, limonoids, citric acid, and other compounds [133]. Grapefruit seed extract can effectively inhibit various bacteria responsible for food poisoning and has antioxidant properties. The presence of active substances such as polyphenols, tocopherols, citric acid, ascorbic acid, and numerous others is responsible for these biological actions. When applied to actual foods, grape seed extract has a wide range of antibacterial activities against various microbial strains. It exhibits strong antimicrobial activity against a variety of foodborne pathogens, such as *Salmonella* spp. and *Listeria monocytogenes* in fresh vegetables, as well as *Candida albicans*, *Pseudomonas aeruginosa*, and *Escherichia coli* O157:H7. Additionally, grapefruit seed extract has demonstrated efficacy in inhibiting *Clostridium perfringens*, which was inoculated into sous-vide chicken products. Foodborne pathogens present in a variety of fruits, vegetables, meats, and seafood can be stopped from growing. The extract also has antifungal activity by causing spore contents to leak and damaging the spore's cell wall and membrane [134-136]. Its application extends beyond direct antimicrobial use in foods; it can also be used as an edible coating or film, such as those made from starch, alginate, or chitosan combined with natural plant antimicrobial extracts. These edible antimicrobial films or coatings help items last longer on the market by acting as barriers against foodborne infections [137].

### 2.9. Date seed extracts

For many individuals residing in dry and semiarid places across the world, date palm is an essential social, environmental, and economic resource [138]. Dates are used to make a wide range of goods, including date paste, marmalade, chocolate, sweet sweets, animal feed, date syrup, and numerous kinds of bread. The construction of boats, the roofing of rural homes, the paper and wood industries, and the

fiber industry all use other date palm byproducts. Fans and straw hats are among the handicrafts made from leaves [139]. However, date seeds have become an environmental concern, with tons discarded daily as waste or mainly used as animal feed [140, 141]. The main constituents of the seed are dietary fiber, protein, carbohydrates, phenols [142], and metals such as potassium, magnesium, calcium, phosphorus, sodium, and iron, which all have biological activities such as antiviral, antibacterial, and antioxidant properties [143]. Additionally, date seeds are a great source of oil that is rich in phenolic compounds, tocopherols, and phytosterols [144-147]. Extensive research on date seed oil reveals its content of vitamins C, E, and beta-carotene, minerals, and fatty acids, making it valuable for food formulations [148, 149], and it offers a promising source of healthful nutrients for humans [150]. Recently, growing interest in the health benefits of date seeds has spurred numerous in vitro and animal studies, along with the identification and quantification of various phytochemicals. With relatively low concentrations of capric, palmitoleic, linolenic, and gadoleic acids, the oil is composed of fatty acids such as oleic, linoleic, palmitic, myristic, and lauric acids [151, 152]. Oils high in oleic acid are particularly valued for their stability and nutritional importance. Oleic acid is regarded as a vital unsaturated fatty acid in foods because of its role in preventing cardiovascular diseases, reducing blood cholesterol, providing high oxidative stability, and providing overall health benefits [153]. Additionally, research shows that lauric acid has antibacterial qualities, a better profile than trans fats, and a protective effect against prostatic hyperplasia [154-157]. Date seed oil is a promising dietary product with demonstrated antioxidant effects, especially if extracted through eco-friendly and contamination-free methods. Research shows that slow pyrolysis of date seeds results in the production of biologically active compounds such as triterpenoids and steroids. Owing to its stearic, palmitic, and oleic acid contents, this oil can be used to formulate anti-inflammatory medications. Its main active constituents are enhancers that promote the absorption of nonsteroidal anti-inflammatory drugs through the skin [158]. These studies highlight how crucial it is to incorporate antioxidant-rich natural materials into a variety of matrices to create antimicrobial films that work well for food packaging. Using date seed extracts, polymeric films containing carboxymethyl

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chitosan and carboxymethyl starch were prepared and assessed as antimicrobial agents against a variety of microbial species. With testing intervals of 15 h, 12 h, 18 h, 15 h, 21 h, and 24 h, the films notably demonstrated considerable inhibition zones, ranging from  $22 \pm 0.49$  mm to  $35 \pm 0.76$  mm, against bacteria such *E. coli* O157, *S. typhimurium*, *L. monocytogenes*, *S. aureus*, *R. oryzae*, and *A. niger* without causing any harmful effects. The study recommended using such materials for packaging fruits and vegetables [159]. Alginate, a widely used marine polysaccharide, has become an attractive polymeric support because it is abundant, inexpensive, nontoxic, biocompatible, nonimmunogenic, biodegradable, and stable; it also has good emulsifying and film-forming properties and is used for packaging. Alginate films have a glossy appearance, are water-soluble, tasteless, odorless, and have good oxygen and grease barrier properties. Several food products, such as fruits, vegetables, meat, fish, and cheese, have benefited from the successful application of these alginate-based coatings [160]. Effective antioxidant films for food packaging made from alginate combined with date seed extracts were reported by *Khwaldia et al.* [159]. The inclusion of date extracts increased the water vapor barrier characteristics, tensile strength, and elongation of the films. In summary, date seeds, rich in active compounds, can be used to produce biodegradable coatings and films with improved functionality and environmental benefits. Preliminary results demonstrate their effectiveness in preserving different food items. Nevertheless, further research is necessary to optimize formulations, functional properties, and sensory acceptance [161, 162].

### 3. Functionalized antimicrobial PVC films

PVC is one of the most widely used plastic packaging materials for food packaging [163] because it readily produces desirable features and offers many benefits, including low cost, wide availability, high flexibility and strength, ease of heat sealing, chemical inertness, high permeability, and excellent self-sticking properties [26]. According to *Leadbitter*, a thorough analysis of PVC use in food packaging applications includes information on the primary chemicals used as well as regulatory frameworks for additive selection that consider waste management, environmental concerns, safety, and health [163]. However, PVC production raises significant environmental issues, especially during the

synthesis of 1,2-dichloroethane, which produces toxic dioxins. To address this problem, biobased approaches have been developed that convert bioethanol from sugarcane into bioethylene, which can then be used to produce vinyl chloride monomers via traditional organic methods. These materials offer important barrier properties against oxygen and water vapor, making them suitable for storing specific food products. PVC has permeability values of  $1-2 \times 10^{13}$  cm<sup>3</sup>·cm/cm<sup>2</sup>·s·Pa for water and oxygen, respectively, and has a tensile strength of 56.5 MPa and an impact strength of 0.91 J/cm, with a thermal stability characterized by  $T_g = 80^\circ\text{C}$  and  $T_d = -250^\circ\text{C}$  at room temperature [164-167]. PVC films are used for meat packaging, such as poultry and raw fish, to extend shelf-life and retain moisture due to their good thermoforming capabilities [168]. They are also employed as standard materials for packaging fruits and vegetables, including stored bananas (cultivars Saucier), strawberries, apples (cv Fuji), broccoli, mushrooms, and sapota, owing to their ability to reduce dopamine content, retain aroma, and lower respiration rates [169-171]. Nonetheless, the nondegradable nature of PVC limits its use as a packaging material, and surface modifications are often needed to reduce contamination risks and meet the demands of effective packaging materials. PVC combined with silver nanoparticles has been tested in chicken and turkey meats at various concentrations, times, and temperatures, with silver migration into food matrices remaining within permissible limits (0.03–8.4 mg/kg) [172, 173]. Similarly, a highly antimicrobial PVC film was developed by bonding PVC to polyhexamethylenediamine guanidine, followed by the grafting of mercaptopropyl trimethoxysilane and aminopropyl triethoxysilane with glutaraldehyde as a crosslinker. This biofilm effectively combats bacteria through direct contact [174]. We introduced new antimicrobial materials, incorporating Cu(I) and Cd(II) complexes of bisacylthiourea derivatives, into PVC films. Compared with standard antibiotics, the PVC/Cd composite showed significantly superior antibacterial activity against resistant species, outperforming the PVC/Cu variant. These materials could be adapted with minor modifications for use in food packaging as a novel approach to antimicrobial surface engineering in the food industry [175]. Films containing organic or inorganic antimicrobial agents such as EDTA, fungicides, parabens, and other chemicals have been

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developed for food packaging because of their ease of production and high performance [176-181]. For example, PVC-based films with quercetin and silver nanoparticles were highly effective at inhibiting bacteria such as *E. coli*, *S. Typhimurium*, and *L. monocytogenes* [182]. Assis *et al.* [181] recently examined the antimicrobial activity of PVC-silica ( $\text{SiO}_2$ )/AgNPs composite films for papaya packaging and reported the complete elimination of *S. aureus*, *E. coli*, and *Penicillium funiculosum* after 24 hours. Grafting copolymers onto PVC urinary catheters improves biocompatibility and provides binding sites for lysozyme, reducing bacterial adhesion and biofilm formation—lysozymes reduce *Staphylococcus aureus* adhesion [183]. Extracts from *C. reinhardtii* exhibit antimicrobial activity against bacteria and fungi, suggesting their potential for antimicrobial packaging applications. [26] Blending algal extracts with PVC yields bioplastic films that are promising eco-friendly alternatives to conventional plastics [26]. Algal biomass contains bioactive compounds, including lipids, proteins, carbohydrates, and fatty acids, making it a valuable source of biodegradable plastics [184, 185].

### 4. Application of antimicrobial PVC films in food packaging

To improve food safety, prolong shelf-life without compromising quality, and prevent certain bacteria from growing on food, antimicrobial packaging is essential. When the microbiological count reaches  $10^7$  CFU/g to  $10^8$  CFU/g, which is the standard for shelf-life indication, food is deemed ruined. The incorporation of essential oils, plant extracts, enzymes, chitosan, and bacteriocins into natural antimicrobial packaging has been the subject of numerous investigations [186]. By providing consumers with crucial information on food freshness and spoilage, the incorporation of natural antimicrobial agents into packaging materials enhances packaging technology [187]. Today, customers want foods that are free of chemicals and preservatives, increasing the demand for natural antimicrobials to purify food and increase shelf-life. Many natural chemicals with a broad antibacterial spectrum against a wide range of microbes can be found in plants, herbs, and spice extracts [188]. The chemical stability, kinetics, and mechanisms of action of these natural antibacterial agents are still unknown, although they also

possess antioxidant properties used in some medications. Environmental factors affect an enzyme's antimicrobial activity; for example, lysozyme is extremely sensitive to pH and temperature, which can reduce its effectiveness against Gram-negative bacteria. Bacteriocins are small molecules produced by bacteria that inhibit the growth of similar or closely related strains. Edible films, coatings, and plastic wraps are directly treated with certain bacteriocins, such as lactacin, nisin, and EDTA [11]. Other products, such as pediocin and propionacin, are incorporated into food or packaging systems to prevent microbial growth. The bacteriocins produced by live bacteria during food fermentation are added to food packages as probiotics to increase their antimicrobial properties. Immobilized bacteriocins, such as nisin and lactacin, are incorporated into polyethylene or polyamide pouches to protect against *Lactococcus lactis*, *Listeria innocua*, and *Staphylococcus aureus* in refrigerated cheese and ham, thereby extending shelf-life [189]. In addition to interacting with food, the active ingredients in packaging help protect the area between the food and the package [190]. Adding active substances to natural and synthetic polymers through coating or film development is a useful method for extending the shelf-life of food. The physical and mechanical properties of the polymer, as well as the film thickness, affect the effectiveness of antimicrobial packaging. Notably, the hue and opacity of polymers can be altered by the addition of plant extracts [191]. The characteristics of polymers are also altered by the addition of antimicrobial agents [192]. By altering the polymer structure, which influences diffusion or initiates direct interactions with antimicrobials, polymer additives such as stabilizers, plasticizers, lubricants, and fillers can adversely affect antimicrobial activity [193]. The chemical composition, mechanism of action, spectrum of activity, bacterial growth rate, and physiological conditions of the target microorganisms are among the variables that affect the integration of antimicrobial drugs into the polymer matrix. Particularly important are diffusion kinetics, which dictate the release of antibacterial chemicals from the polymer [194].

To obtain a PVC antimicrobial material, PVC must be modified to reduce contamination risk when used in food packaging. For food packaging, PVC needs to be plasticized with plasticizers (up to 30%), and palm oil olein, a nontoxic



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edible triglyceride, must be attached to the PVC backbone to create a suitable antimicrobial film for packaging applications [195]. Attaching a biocide agent to the surface is one approach to produce an antimicrobial film [196], as demonstrated by incorporating antibiotics such as nisin or triclosan into PVC products [197, 198]. Sodium ampicillin, an antibiotic, was successfully incorporated into PVC from a DMF solution, and the resulting film had antibacterial properties against *P. aeruginosa*, *K. pneumoniae*, *S. aureus*, and *E. coli*. The DMF film shows strong antibacterial activity due to the ease of antibiotic access afforded by its morphology within the PVC matrix [199]. Additionally, triclosan was incorporated into PVC [200], and its antibacterial efficacy against *S. aureus* and *E. coli* was examined and compared with that of real PVC sheets containing silver. The results highlighted the importance of the hydrophilicity of the PVC surface for bacterial adhesion. Highly antimicrobial PVC films were prepared by blending equal parts of PVC and silkworm cocoon waste (1:1 w/w) and using moringa seed oil as a biobased plasticizer, with or without silver nanoparticles [199]. Another plasticizer derived from soybean oil and glycerol, a formal vegetable oil integrated into PVC, has shown sustainable and eco-friendly plasticizing properties and considerable antibacterial efficacy against common infections, including *Staphylococcus aureus* and *Escherichia coli*. The resulting film was suitable for food packaging purposes [201]. The effects of nanoclay and an active agent, catechin lysozyme, on PVC-based film properties were also studied. The microbial assessment revealed that the composite film had 5.74 log CFU/g after 7 days of storing pork meat. For yeasts and molds, a similar count of 6. A total of 6.82 log CFU/g was observed on the PVC film.

### 5. Migration of chemicals into food matrices from packaging materials

When chemicals such as plasticizers, solvents, and stabilizers move from packing materials into food, it is referred to as migration. This can occur because of physical, chemical, or environmental factors and may affect consumer health and food safety [202]. Thus, ensuring food safety and fulfilling regulatory requirements requires an understanding of migration [203]. Plastic components, inks and coatings, additives, plasticizers, and antioxidants are examples of

chemical substances found in packaging materials. Numerous physical and chemical factors affect the migration of chemicals from food packaging into food products. Temperature is a crucial factor; higher temperatures can accelerate migration by encouraging the chemicals in the packaging to diffuse into the food. This is especially crucial for storage and transit under less-than-ideal circumstances. Various processes, including migration, leaching, and chemical reactions, can transfer contaminants from packaging materials to food when the packaging materials come into direct contact with food. The main mechanisms of migration include diffusion [204], which is the most common mechanism; volatilization [205], which occurs when volatile chemicals in the packaging evaporate; permeation [206], where small molecules pass through the packaging material itself; convection [207], which involves the movement of chemicals such as gases or liquids in packaging; and chemical reactions [208] between the food and the packaging materials. Interestingly, package structural elements, such as multilayered films, can serve as barriers, restricting chemical diffusion and lowering migration rates. There is no relationship between migration and the presence of recycled substances. International organizations such as the food and drug administration (FDA) and the European food safety authority (EFSA) established guidelines for choosing simulants and testing parameters [209] to ensure that migration tests accurately represent real-world conditions.

### 6. Regulations of natural antimicrobials in the food industry

International regulations strictly regulate food additives [210], yet nations frequently dispute whether additions are safe, what amounts are allowed, and what applications are permitted. For example, only a small number of substances, primarily organic acids, are now authorized as food preservatives in Europe. To license food additives for human consumption, a rigorous procedure is followed. When requesting approval for a novel additive, an applicant gives comprehensive scientific safety information, together with a formal request to the European Commission, the EU's executive branch. After the application is accepted, the Commission requests that the European Food Safety Authority (EFSA) examine whether these substances are safe for use. In

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addition to reviewing current additives with new scientific data or evolving regulations, the EFSA also assesses the safety of novel additives before approval [211]. Comparably, in China, the only industry group with the authority to assess and assist the Chinese government in regulating food and food additives is the China Food Additives Association (CFAA) [212, 213]. In the United States, the Food and Drug Administration (FDA) evaluates unapproved food additives for safety before approving them. This evaluation considers the usual intake, possible immediate and long-term health consequences, and additional safety considerations. The FDA has regulatory authority over food additives, as stated in its Guidelines for Industry. Once approved, the FDA issues regulations that specify the types of foods it can be used in, the maximum allowable quantities, and proper labeling, all of which are outlined in Title 21 of the Code of Federal Regulations. However, under FDA rules, the Environmental Protection Agency (EPA) has established guidelines for pesticide compounds and residues in food. Additionally, the FDA regulates antimicrobials used in food packaging as food additives and does not classify them as "pesticide chemicals" [214].

### 7. Obstacles and restrictions in the use of natural antimicrobials in the food industry

The growing customer desire for chemical-free food items has prompted food firms to use natural substances, as studies have demonstrated the broad and promising effects of natural antibacterial agents. Natural antimicrobials have drawbacks and limitations, including effects on sensory attributes such as flavor, color, and texture [215]. Since these compounds are thought to be safer and more ecologically friendly than synthetic chemical preservatives, regulatory and safety concerns [216] are receiving increased attention. Although many natural antimicrobials have received GRAS designation, regional differences in food safety regulations have caused inconsistencies in the licensing of specific compounds, allowable concentrations, and food applications. Certain natural antimicrobials can cause allergic reactions, adverse effects, or disrupt gut flora if used excessively or for a long time. The addition of natural antimicrobials can also alter the flavor, aroma, and appearance of food. To solve these issues,

more research is necessary to develop methods for isolating and purifying natural antimicrobial agents, assessing their safety, and creating consistent regulatory frameworks for their safe and effective use. Significant challenges in preserving antimicrobial efficacy also arise from stability and shelf-life, as many bioactive substances break down quickly in the presence of environmental factors such as light, heat, oxygen, and pH. Due to their volatile nature, essential oils often lose effectiveness during processing and storage, thereby decreasing their ability to inhibit microbial growth. Novel delivery methods have been developed to protect natural antimicrobials from environmental degradation, preserving their bioactivity and extending their shelf-life. These methods include edible coatings, nano-encapsulation, and microemulsions. Since extracting natural antimicrobials often involves costly, time-consuming, and technically complex processes, cost and scalability remain major barriers to widespread use, limiting large-scale industrial production. Geographical and seasonal variations in the availability of raw materials make standardization even more difficult and affect the reliability of the supply and the efficacy of the final product. In price-sensitive markets, natural antimicrobials are less viable because of their high production costs. To overcome these obstacles and promote the commercial application of natural antimicrobials, cooperation between academic institutions, industry stakeholders, and regulatory bodies is essential [39]. A synergistic approach with other preservation technologies is thought to be advantageous to prevent adverse effects from high concentrations of these substances [42]. To enhance the use of these antimicrobials in food systems without harming the organoleptic properties of food products, further research on extraction methods, application strategies, and optimal dosages is necessary [46].

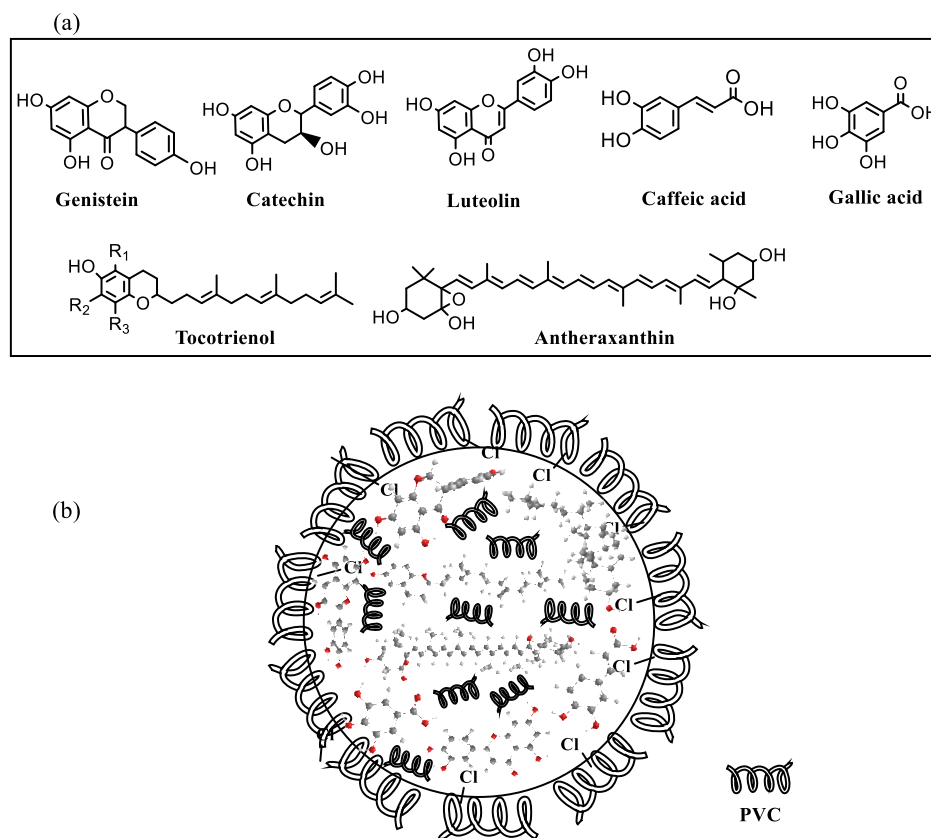
### 8. Conclusion

Applications for antimicrobial compounds in food, particularly food packaging, have been around for some time. These include extending the shelf-life of packaged food and preventing the growth of germs. The chemical composition, mechanism of action, range of activity, rate of bacterial growth, and physiological conditions of the targeted microorganisms are among the parameters that influence the incorporation of antimicrobial compounds into the polymer matrix. The production of natural antimicrobial films involves

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either direct coating or the incorporation of synthetic or natural antimicrobial agents into the film. Several natural antibacterial agents, such as essential oils, bacteriocins, lysozymes, organic acids, chitosan, grapefruit seed extract, AITC, and the primary flavoring ingredients found in cruciferous plants, date seeds, and polysaccharides, are integrated into food packaging films. Although many natural antimicrobial agents have received GRAS designation, cost and scalability remain important challenges to increasing their use. Geographical and seasonal variations in the availability of raw materials make standardization even more difficult and affect the reliability of the supply and the efficacy of the final product. In price-sensitive markets, natural antimicrobials are less viable due to their high production costs. In this work, we introduced date seeds as promising, inexpensive, and safe possible prototypes for creating antimicrobial food packaging films using these well-known antimicrobial agents. The primary components of date seeds include fiber, protein, carbohydrates, phenols, and

metals; these substances have a variety of biological properties, including antiviral, antibacterial, and antioxidant properties. Furthermore, dates are used to make a wide range of goods, including date paste, marmalade, chocolate, sweet sweets, animal feed, date syrup, alcohol, and several kinds of bread. In addition to being a good source of oil that is high in phenolic compounds, tocopherols, phytosterols, date seeds are also useful for food formulations because of their content of minerals, fatty acids, beta-carotene, and vitamins C and E. Thus, the use of fruit byproducts to create antimicrobial films is a great way to repurpose these residues, utilize their bioactive chemicals, reduce disposal issues, and support the circular economy concept. Thus, a natural product that is rich, valuable, and effective can be used to create biodegradable films and coatings that have improved functionality and environmental benefits. However, further research is needed to enhance formulations, functional qualities, and sensory acceptance and to determine the GRAS designation.



**Figure 10.** (a) Representative chemical structures found in date seeds, (b) a proposed model of a PVC film containing several oil constituents found in date seeds.

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One of the earliest polymers used in food packaging was PVC, which replaced many traditional materials like glass and various thermoplastics. Owing to its exceptional cost/performance ratio, ease of printing, and compatibility with a variety of additives due to its polar nature, PVC is a great choice for preserving food freshness and extending shelf-life. It can also block gases such as oxygen and water vapor. PVC has worldwide approval for use in food contact applications, and many PVC/additive blends are already listed on European incomplete additive lists, including those outlined in EC Directive 2002/72 and its subsequent amendments. Blending various extracts with PVC yields bioplastic sheets, which are promising eco-friendly replacements for traditional plastics. Generally, using plasticizers is essential to make flexible PVC biofilms for food packaging, and many well-documented PVC plasticizers are available. Notably, because of the potential presence of different chemicals and their varying migration rates, Zero Waste Europe recently advised against using PVC in contact-sensitive applications like food packaging. However, removing PVC from packaging will restrict consumer choices without providing any additional environmental benefits. This study aims to differ from the general literature by addressing recent advances that incorporate various natural antimicrobial agents, which are known to be safe for the food industry, into the PVC matrix to create effective antimicrobial films and their application in food packaging. This work provides a thorough summary of recent studies in antimicrobial food packaging, with a focus on the application of antimicrobial agents and PVC-based films. Date seeds are both nutritious and therapeutic, and making bioactive films from their byproducts is a promising way to use them and lessen their disposal issues. A proposed model of a PVC film containing representative chemical structures, such as phytosterols, tocotrienols, carotenoids, flavonoids, phenolic acids, and phytoestrogens contained in date seeds, is shown in Figure 10. The use of the developed films to inhibit and prevent the growth of spoilage microbes during conservation and to extend the shelf-life of stored food will be reported soon.

## Author contributions

H. H. A. M. H.: Conceptualization, methodology, resources, supervision, writing—review and editing.

E. E.: Data curation, investigation, methodology, validation.

A. E.: Data curation, investigation, methodology, validation.

N. E. K.: Resources, data curation, methodology, original writing.

All authors have read and agreed to the published version of the manuscript.

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The authors declare no conflict of interest.

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## Data availability

Not applicable.

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