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REVIEW PAPER

Application of Natural Antimicrobial Agents in Different Food Packaging Systems and Their Role in Shelf-life Extension of Food: A Review

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Abstract
In the present era, using natural antimicrobial agents has gained a wide recognition by consumers and the food industries alike, due to their safe nature as compared to the synthetic antibiotics that cause resistance in the microbes. These compounds have a potential to be used against broad range of microbes and food pathogens. There is a wide range of antimicrobials derived from plants, animals, and microbial sources as well as from the byproducts of food processing industry. The antimicrobial agents may be applied either directly in contact with the product or by indirect contact systems (as films, coatings, sachets and or encapsulated) of packaging. The mode of diffusion varies from one system to the other. Therefore, different packaging systems have different applications as per the requirement and or situation. The incorporation of antimicrobial agents in food packaging systems have been found to enhance the keeping quality in addition to the shelf-life extension of the food products. Despite the growing demand of the natural antimicrobials, there are certain limitations like objectionable flavors, authoritarian issues, undesirable interaction with the food components, and non-optimized standards associated with them. Hence, there are still many research gaps to be filled for commercializing or industrial applications of various types of antimicrobial agents. This article provides information about the antimicrobials from these sources along with their prospective applications in the food systems. Besides, a complete insight of their mode of application for the shelf-life enhancement in food products is also outlined.

Keywords Edible · Essential oils · Extract · Packaging · Shelf life · Spoilage

Introduction

Food spoilage is a major area of concern for food industries incurring heavy economical losses. Spoilage of food may be any sort of physical dent or chemical damage or even both. Chemical spoilage of foods includes oxidation, color change, metabolic degradation and microbial contamination resulting in development of off odors and off flavors [138]. Outbreak of food borne illnesses is a distressing and worrisome issue faced by consumers, food safety establishments and food industries that is majorly caused by the bacterial intoxications and contaminations. To curb these contaminations and safe food production, several methods including thermal treatments, non-destructive non thermal processing methods (Pulse electric field (PEF) processing, Plasma treatments, irradiation, and modified packaging methods), water activity reduction and addition of preservatives are used (Fu et al. 2016) [73]. However, considering the old practices of food preservation, chemical preservatives like synthetic antimicrobials and thermal treatments are majorly employed to produce safe food products [16, 138]. The chemical antimicrobials include organic acids and their salts like benzoic acid, propionic acid, sorbic acid, citric acid, tartaric acid, sodium benzoates, sulphates, nitrates, sorbates etc. [48, 102]. However, due to the consumer awareness and the

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safety regulations about the injurious effects (carcinogenic, toxicity, teratogenicity) of these chemicals, these are in limited usage in present times [16]. Owing to ill effects and more importantly the development of resistance in microorganisms against these chemical antimicrobials, substantial efforts are being made for the extraction and processing of natural bioactive compounds that can be an alternative to the synthetic antimicrobials for safe shelf-life extension of food products [79]. Natural antimicrobials can be derived from various naturally occurring sources like plants, animals, microflora (bacteria, fungi like mushrooms etc. [72, 102]. Some of the important natural antimicrobials include lysozymes from eggs, lactoferrin (glycoprotein), flavonoids and phenolic compounds from fruits and vegetables, tannins, saponins, and essential oils from spices and herbs, Chitosans from crustacean wastes, bacteriocins from lactic acid bacteria etc., [72, 73], [102], 119.

Application of antimicrobials to food products can be done in various forms, like direct inclusion into the food systems, or incorporation into the packaging films or coatings as antimicrobial preservative releasers [72], [106, 118]. Natural antimicrobial agents can also be used to introduce new and improved products in the market in addition to their role in shelf-life enhancement of existing products [16], 109. This descriptive review explains different antimicrobial agents derived from various natural sources, their mode of application, limitations, challenges and future prospective of their usage in compliance of food safety and preservation.

Natural Antimicrobial Agents and Their Sources

A shift of focus from use of synthetic antimicrobial agents to natural antimicrobials is due to constant awareness of consumers about the ill effects of the synthetic antimicrobials and a dreadful antibiotic resistance associated with them. Natural compounds and primarily the secondary metabolites tend to be an important source of drugs in medical field today. These antimicrobials can be of plant source, animal source or microbial source (Fig. 1). Secondary metabolites of animal origin form a major group of natural antimicrobial agents. Natural antimicrobials from animals can be categorized as chelators, enzymes, immunoglobins etc. Lactoferrin, is generally found in milk, saliva, tears etc. and acts as an iron chelator, that makes it an effective antimicrobial against several pathogenic bacteria and viruses including food borne pathogens like *E. coli, Carnobacterium, Klebsiella, Listeria* sp. etc. [72]. Likewise, conalbumina a glycoprotein present in egg white poses both bacteriostatic as well as bacteriocidal effect against many pathogenic microorganisms including *Streptococcus mutans, S. aureus, Proteus* spp, *E. coli* etc. by disturbing the metabolic pathway of these microbes due to its capacity of binding with ferric ions involved in electron transport chain [88]. Chitosan, a natural polycationic biopolymer that is a derivative of crustacean exoskeleton acts as a natural antimicrobial against gram positive

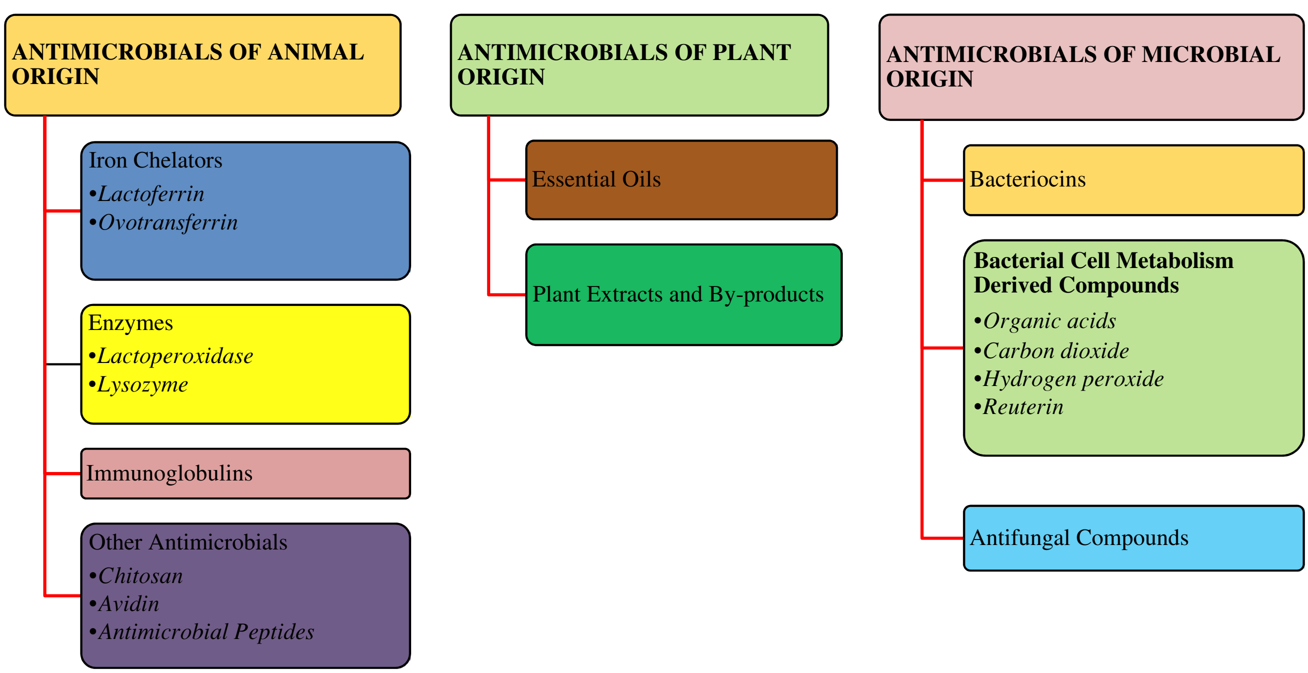

Fig. 1 Natural antimicrobial agents from different sources

and gram-negative bacteria including *S. typhimurium, S. aureus, B. cereus, E. coli* etc. [72].

On the other hand, the secondary metabolites derived as antimicrobials from plants include essential oils extracted from seeds (olive, fennel, parsley, caraway etc.), bulbs (Garlic, onion etc.), or pods (cardamom) of various plant products [16], from essential oils of peels from citrus fruits (Orange, Lemon, Lime) [34].

Essential oils tend to seep into the lipid bilayer of the microbial structure that leads to leaching of the cell constituents creating disturbance of protein motive force, electron transport, and membrane protein interaction causing structural and functional, degradation of microbial cells, that ultimately leads to cell lysis [132]. Similarly, microorganism produce inhibitory factors that possess antagonistic effect against the pathogens in their proximity. The inhibitory factors produced by the microorganism either possess broad or narrow spectrum of antimicrobial activity against host of microorganisms [12]. Although, there has been a significant characterization of various bacteriocins, still Nisin is considered as most commercially explored bacteriocin, owing to its high heat stability. Nisin acts against gram positive bacteria like *Leuconostoc, L. monocytogenes, C. botulinumetc* [16], and leads to damage in the cell integrity of these microorganisms by attaching to their cell wall [11]. Some other bacteriocins are pediocin, *Sakacin, Lacticin,* out of which pediocin is closer and comparable with the antimicrobial activity of nisin, by acting against *Enterococcus, Lactobaccilus, Clostridium* etc. and specifically causes damage to *L. monocytogenes* Various plant-based compounds and their antimicrobial effects against different pathogenic microorganisms are presented in Table 1.

Shelf-Life Enhancement of Food by the Application of Antimicrobial Agents

Antimicrobial systems come into existence by considering several parameters including the key factors that are accountable for the antimicrobial nature and the type of the food in which the system is to be employed [31]. All these factors are fundamentally responsible for the interaction of anti-microbial system with the microbes in the food matrix. Diffusion of these antimicrobials into food depends on their volatility and the non-volatile movement involves diffusion of active factors responsible for the antimicrobial activity from package, surface of the food or the layer that is in contact with the food. Additionally, the diffusion of antimicrobial compounds depends on release of such compounds into food systems that is arbitrated by the package headspace and is characterized by temperature and the interaction between packaging material and the compounds [89]. The ever-growing interest in application of natural antimicrobials for food preservation has paved a way for extraction and purification of the bioactives that are found in antimicrobial rich raw materials [131]. Extraction and purification of these antimicrobial bioactive components result in standardized compounds that can utilized further.

Incorporation of antimicrobial systems to the food matrix involves either direct application to the food involving a true contact between food matrix and the antimicrobial system or indirect application that does not involve any contact with the food matrix [133]. During the indirect contact system, a mediating agent (essentially a package or a part of it) is used to transfer the antimicrobials to the food product. Such type of packaging systems evolved the idea of active packaging systems. Whereas, in direct contact system, antimicrobial agent is brought into contact of the product by spraying, washing, or mixing. Based on mechanism of antimicrobial actions, active packaging has been classified into following categories.

i. Sachets are used inside the packaging systems that release the antimicrobials in the headspace
ii. Antimicrobials are incorporated on the surface of packaging that allows their migration into the food system
iii. A controlled release of the antimicrobials from the surface of packaging that have these compounds immobilized on them
iv. Use of materials with innate antimicrobial property
v. Direct application of the edible films (containing entrapped antimicrobial agents) on the product surface

In following sections, direct and indirect contact systems of natural antimicrobials in food are discussed in detail.

Direct Contact Systems

The direct contact systems may involve volatile or non-volatile compounds or both that are added to the food matrix from where the active compounds migrate via diffusion mode of mass transfer [70]. The direct contact systems include antimicrobial application in the form of powders, fluids, sprays, and essential oils carried in wash solutions [72]. Encapsulation of the antimicrobial compounds into food systems is one of the major and efficient method for retention of biological activity of the components, besides, modulation of release and diffusion of these components in food matrix that enhances the shelf life of the product [133]. Other techniques used for incorporation of natural antimicrobials in food matrix is micro and nano-emulsion techniques [41] Essential oils have been applied either directly as sprays or indirectly as in films, coatings, or encapsulated systems [16]. In rice-based foods, Saeed et al. [108] examined

Table 1 Plant based antimicrobial compounds or extracts and their antimicrobial activity against specific or series of microbes

Compound/extract	Plant source	Antimicrobial activity against	References
Eugenol	Clove	*Bacillus cereu L. monocytogenes*, *S. enetriditis, E. coli*,	Davidson et al. [27]
Cinnamaldehyde	Cinnamon	*Bacillus* spp., *Aeromonas hydrophila*, *E. coli* O157:H7, *C. jejuni*, and toxigenic bacteria and fungi	Vergis et al. [132], Davidson et al. [27]
Carvacrol [5-isopropyl-2-methylphenol]	Oregano	*Bacillus, C. jejuni*, *Enterococcus* spp., *Pseudomonas* spp., *Shigella*, *Aspergillus*, *Rhodotorula* and *L. monocytogenes*	
Thymol [5-methyl-2-(1-methylethyl)-phenol]	Thyme (*Thymus vulgaris*)		
Methyl chaviol and linalool	Sweet basil (*Ocimumbasilicum*)	Many fungi and bacteria like *Bacillus* spp.	
Thujone [4-methyl-1-(1-methylethyl)-bi-cyclo (3.1.0)-hexan-3-one]	Sage (*Salvia officinalis*)	Gram-positive and Gram-negative bacteria	
Borneol, camphene, pinene and camphor	Rosemary (*Rosmarinus officinalis*)		
Essential oil	Various plant sources (such as Coriander, lemongrass, bergamot, marjoram, fennel, bay, ginger, black pepper, and cumin)	Antimicrobial action of different intensity	Calo et al. [16]
Naringin, hesperidin, ascorbic acid, and other organic acids	Extract of grapefruit seed	*E. coli*, *L.* monocytogenes, *S. aureus*	Irkin and Esmer [61]
	Green tea extract	*S. aureus* and *S. mutans,*	
Allylisothiocyanate	*Brassica* family		
Extract	Grape pomace	*B. cereus*, *A. hydrophila*, *Enterococcus faecalis*, *Proetus vulgaris*, *S. typhimurium*, *Salmonella*, *E. coli*, yeasts and molds and other various harmful microbes	Ozkanet al. [95]
Phenolic compound oleocanthal	Olive (juice powder and pomace)	Bactericidal against food-borne pathogens,	Friedman et al. [42]
Ellagitannins	Pomegranate (peel extract)	*E. coli*, *Y. enterocolitica*, *B. cereus*, *L. monocytogenes* and *Pseudomonas* spp.	Kanatt, et al. [65], Li et al. [77]
Chlorogenic acid	Quince (peel extract)	Gram-positive *S. aureus* and Gram-negative *E. coli*, *P. aeruginosa* and yeast *Candida albicans*	
Gallotannins	Mango (kernel extracts)	Food-borne pathogens	Abdalla et al. [1], Engels et al. [37]
Seed extract	Papaya, plum, guava, and tamarind	Gram-positive and Gram-negative bacteria	
Sinapic acid	Mustard seed meal	*P. fluorescens*, *B. subtilis*, S. aureus, and *L. monocytogenes*	Engels et al. [37]
Flavonoids	Almond skins	*S. aureus* and *L. monocytogenes*	Mandalari et al. [80]
Extracts	Hulls of Buckwheat, Pigeon pea, (*Cajanus cajan*) and mung bean (*Vigna radiata*)	Various spoilage causing and pathogenic bacteria	Kanatt et al. [66]

the efficiency of basil, thyme, and oregano essential oils to inhibit *Bacillus cereus*. In meat-based products also, thyme and oregano oil were found to bring an enhancement in their shelf-life [71]. When added in minced meat at 3%, Tunisian sage (*Salvia officinalis* L.) and Peruvian peppertree (*Schinusmolle* L.) essential oils effectively eliminated the populations of *Salmonella* and *S. Enteritidis* during storage under refrigeration for 15 days [53]. Limonene applied on chicken legs and thighs caused complete inhibition of *Campylobacter jejuni* [16]. *Satureja horvatii* essential oil comprised of p-cymene and thymol, when applied to ground pork resulted in the inhibition of *L. monocytogenes* thereby improving its keeping quality [15]. Button mushrooms fumigated with EO's of clove, thyme and cinnamaldehyde maintained their keeping quality throughout 16 days of cold storage [43]. Bay essential oil in combination with MAP, however excluding oxygen controlled the numbers of *L. monocytogenes* and *E. coli* in ground chicken meat in addition to increasing its shelf life [60]. Oil emulsions and vapors of cinnamon leaves were used to control the fugal decay in table grapes after being inoculated with *Botrytis cinerea* by Melgarejo-Flores et al. [85]. The antifungal effect of these essential oils is ascribed to the presence of the principal compounds i.e. cinnamaldehyde and eugenol. Garlic extracts and clove oil were applied in curative and protective modes on three apple cultivars inoculated with postharvest pathogens *Penicillium expansum*, *Botrytis cinerea,* and *Neofabra alba*, either directly or as vapors. The curative mode wherein the antimicrobial was applied post-inoculation was found effective in arresting the decay when the fruit was treated directly with antimicrobials [26].

Siroli et al. [124] created a system for the application of natural antimicrobials through a wash solution to fresh-cut apple and lamb's lettuce. The antimicrobials included lactobacilli as biocontrol agents and hexanal and citral as antagonistic factors against *E. coli* and *L. monocytogenes*. *Lactobacillus plantarum* proved to be an effective bio-control agent accelerating the inhibition of both pathogens in minimally processed apples. The combinatory application of bio-control agents with natural antimicrobials brought about a further enhancement in shelf-life. *Salmonella typhimurium* inoculated on iceberg lettuce was inactivated because of washing with oregano oil. The results produced by wash treatment were comparable enough with generally used disinfection methods [47]. Grape tomatoes treated with a wash solution containing thymol, an active component of thyme essential oil, exhibited a 7.5 log reduction in the population of *Salmonella* maintaining its overall quality throughout a 16-day storage period [78]. Blueberries were treated with a 2% wash solution of lactic acid by spraying, followed by storage at -. The combination of freezing with antimicrobial (by spraying 2% lactic acid solution) brought the count of *S. typhimurium*, *E. coli* and *L. monocytogenes* down to undetectable in blueberries stored for one week at 12 °C [126].

Minimally processed carrots and apples inoculated with *E. coli* and *S. typhimurium* were treated with hydrosols of thyme, sage, black cumin, and rosemary. All the hydrosols were found to be efficient enough in reducing the counts of inoculated microbes, particularly thyme hydrosol, which dominated in terms of the inhibitory effect. The antimicrobial function of lysozyme in combination with EDTA was studied in Mozzarella cheese, the cheese stored for 8 days at 4 °C showed decreased populations of *Pseudomonads* and *Coliforms*. Similarly, Burrata cheese treated with lysozyme and packaged under MAP presented an enhancement in shelf life [30]. Nisin and EDTA alone or in combination rendered fresh chicken shelf-stable for 24 days under MAP at 4 °C [58]. Pisoschi et al. [102] observed that application of combined treatment of lactoferrin and nisin helps in overall quality retention of Turkish meatballs in addition to the reduction of spoilage causing bacteria. Lactoperoxidase, when added to skim milk at 25 °C, prolonged the lag phase of inoculated *L. monocytogenes* from 3 to 50 h this treatment also prevents the growth of *Salmonella*, *S. aureus*, *L. monocytogenes,* and *E. coli* in vegetable juices, beef, and whole eggs [127].

Bactericidal effect of rosemary extract against *L. monocytogenes* was seen in broccoli juice stored at 30 °C for 30 days [49]. Similarly, rosemary extract inhibited *C. jejuni* [101] and cranberry powder inhibited *L. monocytogenes* [135] in meat.

Direct application of antimicrobials plays a pivotal role in curbing microbial deterioration in foods, however, a question regarding the effective concentration of these natural antimicrobials that needs to be optimum without posing any awful effects on the organoleptic quality of food products. To avoid the undesirable effects of high quantity of these compounds, a synergistic effect with other preservation technologies is regarded as favorable [72].

Indirect Contact Systems

Indirect contact system involves immobilization of antimicrobials on the carrier fills that are then brought in contact with the food surface using the edible coatings. From these contact materials, the non-volatile antimicrobial compound diffuses into the food system, while the volatile compound is released into the headspace. Considering the development in the direct contact systems, natural antimicrobial systems are now being used in the form of sachets that carry antimicrobials and micro/nano particles that encapsulate the active compounds. Both the systems are mostly based upon controlled release of volatiles into the headspace and do not necessarily need to be contacted with the food surface. While it always holds true for sachets, micro/nanoparticles can also be added to both solid and liquid food as needed [69]. All these systems have been implored for their successful application in food products as would be highlighted in the upcoming sections.

Packaging Films

In the recent developments of packaging films, bio films are obtained from biopolymers that possess a three-dimensional gel type structure and are used to wrap around the food products. Over the years, these bio films have grabbed a substantial attention ascribing to their eco-friendly nature and GRAS characteristics. Use of these films has increased because of their capability of being used as natural antimicrobial systems [83, 125].

Lacey et al. [28], developed a novel food packaging film for the shelf-life enhancement of hake fillets, the film was made of agar (base material) and active ingredients (probiotics and extract of green tea). The film was found effective in reducing spoilage microorganisms like *Shewanella putrefaciens* and *Photobacterium phosphoreum* (Beneficial effects observed in terms of increased shelf-life and reduction of spoilage microorganisms were attributed to the antimicrobial efficacy of green-tea extract against bacteria. Green tea polyphenols, principally catechins and (−)-epigallocatechin-3-gallate are primarily responsible for inhibiting microbial growth through many different mechanisms [25]. In a similar investigation conducted by Priyadarshi et al. Priyadarshi, Kim and Rhim, [103], LDPE (linear low-density polyethylene) film co-extruded with grapefruit seed extract (GFSE) was used in preservation of beef. The study observed that the addition of antimicrobial agent (GFSE) in LDPE resulted in shelf-life extension of the beef by a week. Jang et al. Jang et al. [62] reported that rapeseed-protein and gelatin film when applied to strawberries containing GFSE stopped the growth of *L. monocytogenes* and *E. coli* O157:H7. Other studies have found that one decimal reduction in pathogenic bacteria (*Listeria monocytogenes*) can be achieved by the incorporation of GFSE in a soy protein-based films [125].

A polypropylene (PP) film layered with oregano essential oil depicted significant reduction of *E. coli*, *S. enteric* and *L. monocytogenes* in salad kept at 4 °C for two days. Sprouts stored at 10 °C showed decreased microbial flora because of allyl isothiocyanate (AITC) carried by oriented polypropylene/polyethylene (OPP/PE) film [110]. Garlic essential oil incorporated in plastic film significantly inhibited the microbial growth on sprouts and was also found to have inhibitory action against *S. aureus* and *B. cereus* in an alginate film (Pranoto, Rakshit, and Salokhe, 2005). The incorporation of essential oil of oregano, rosemary, and garlic into a whey protein isolate (WPI) based films resulted in the inhibition of *S. aureus*, *E. coli*, *L. monocytogenes* and *Salmonella enteritidis* [7]. Similarly, milk protein-based films carrying oregano essential oil are found to be effective in reducing the spoilage microorganisms in meat during storage at 4 °C for 7 days [95]. Chicken breasts wrapped in films containing carvacrol exhibited a reduction in the growth of *E. coli*, *S. enterica* and *L. monocytogenes* when stored at 4 °C for 72 h [49]. Citral incorporated in kafirin (sorghum protein) based films had prolific antimicrobial action against *C. jejuni*, *L. monocytogenes* and *P. fluorescens* [44]. An edible film elaborated from strawberry puree carrying carvacrol and methyl cinnamate as antimicrobial agents were used to preserve strawberries contained in clamshells. The outcome of using such films was observed as a reduction in the appearance of any kind of visible decay. Moreover, the strawberries maintained their appearance and texture better than the untreated ones [99]. Ayala-Zavala et al. [8] used a bio film incorporated with cinnamon leaf oil for preserving the quality of fresh cut peach. In beef patties, Emiroglu et al. [36] demonstrated the antimicrobial efficacy of oregano and thyme EO's incorporated in soy protein-based films. Antimicrobial films containing 1.5% oregano EO demonstrated significant action against pseudomonas and other microflora on fresh beef [40]. A novel nanocomposite film based on thermoplastic starch/clay (TPS/MMT/EOC), containing thymol and carvacrol was applied as a potential antimicrobial inside PET containers for strawberries. After inoculation with *Botrytis cinerea* causing gray-rot, the fruit was stored at room temperature for 5 days. The growth of fungi and consequent spoilage was evident in untreated strawberries whereas for the antimicrobial added package, strawberries were intact in terms of quality attributes as well as the incidence of rot was minimized (Fig. 2) [17]. Incorporation of antimicrobial agents to polymeric matrices results in changes of their physical and mechanical properties. A brief account of effect of different antimicrobial agents on various polymeric matrices is given in Table 2.

Chitosan, possessing inherent antimicrobial activity has also been applied for attaining enhanced and safe shelf-life of foods. Chitosan coated polypropylene films when applied to Kashar cheese acted as antagonist towards spoilage microflora during 14 days of storage under vacuum, bringing considerable reduction in microbial count as compared to control cheese. Chitosan in combination with oregano and clove essential oils acted synergistically in reduction of pathogenic microorganisms in bologna [35]. Similar results were observed in vitro against *L. monocytogenes*, *E. coli*, *S. aureus* and *S. typhimurium* employing chitosan film carrying *Thymus kotschyanus* essential oil as an antimicrobial agent. Starch-chitosan composite coated on minimally processed carrots acted as an effective inhibitor of microbial growth consequently improving the safety and stability of the product.

Bacteriocins, a class of antimicrobials obtained from bacterial synthesis form a widely preferred choice in the

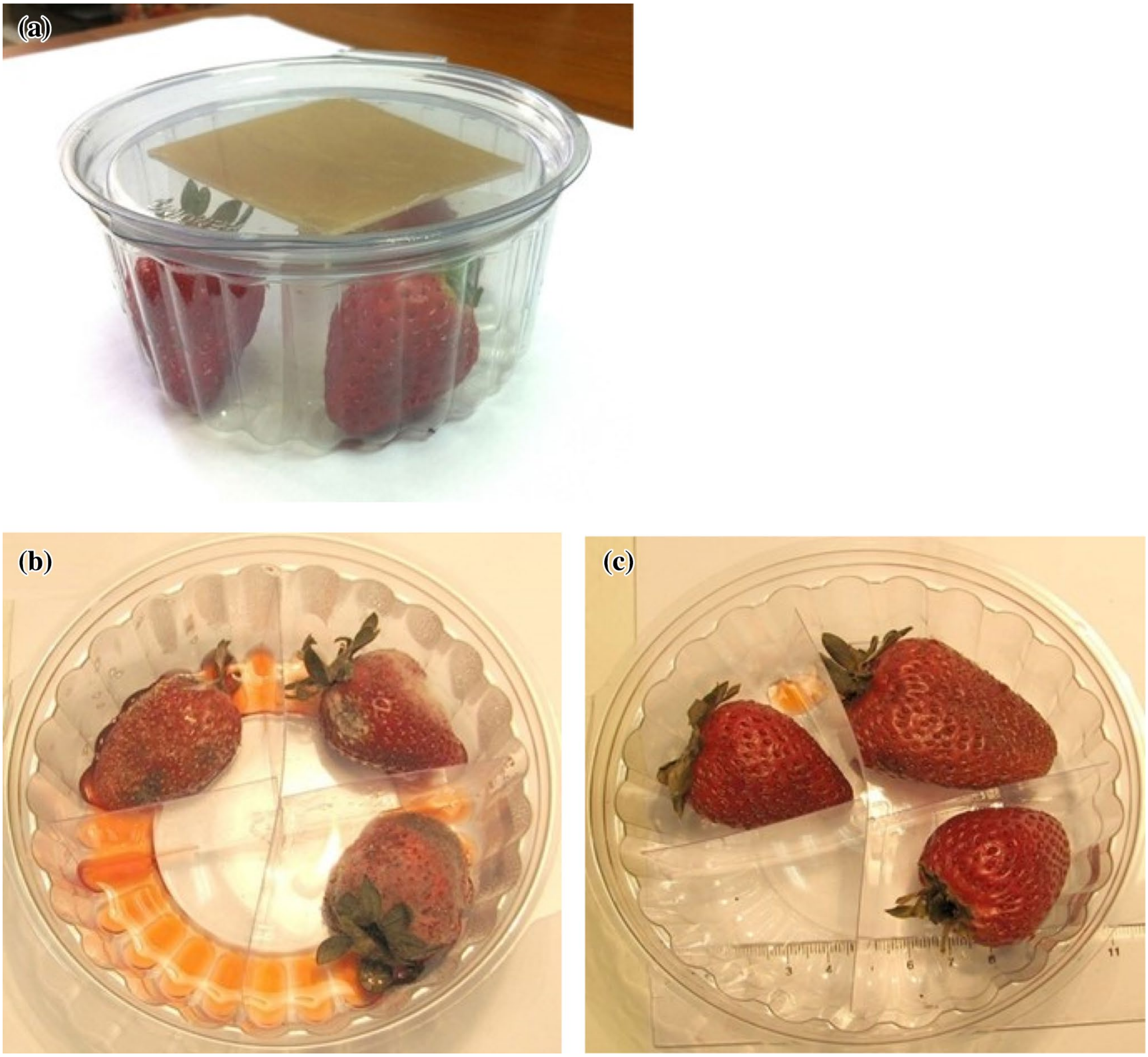

Fig. 2 (**a**) Test packaging used for In vivo antimicrobial assays of strawberries inoculated with *Botrytis cinerea*- in test package (**b**) Packed Fresh strawberries devoid of nanocomposite film control strawberries packed without nanocomposite film, and (**c**) Packed strawberries along with TPS/MMT/EOCs nanocomposite film [17]

domain of natural antimicrobial packaging given their thermal and acid tolerance. Numerous studies carried out by different researchers report the antimicrobial efficacy of these bacteriocins when applied to food systems. Cellulose based film, added with pediocin demonstrated an inhibitory response towards *L. innocua*on sliced ham for 15 days storage at 12 °C whereas films without pediocin failed to inhibit microbial growth [18]. In an in vitro study carried out by Massani et al. [82], lactocin obtained from lactic-acid bacteria added to multilayer-plastic films presented inhibitory effect against *L. plantarum* during the storage period of 7 days at 5 °C. Antibacterial peptides obtained from *Bacillus licheniformis* carried in a film applied onto paneer effectively reduced the population of *L. monocytogenes*, because of slow diffusion of the peptides into the food matrix [91]. A film incorporating natamycin at 2 and 4% caused satisfactory inhibition of *P. roquefortii* in Gorgonzola cheese [116]. Kuorwel et al. [76] studied the antimicrobial effects of nisin, through different types of films on food products. Significant inhibition of *L. monocytogenes* in hot-dogs,*S. typhimurium* on boiler drumstick skin and reduced microbial load in ground beef and oysters was recorded. In mini red Baybel cheese, sodium caseinate film on which nisin was coated immensely decreased the counts of *L. innocua* after

Table 2 Effect of antimicrobial agents on packaging systems

Antimicrobial agent	Effect on packaging system characteristics	References
Thyme essential oil	Addition of 0.5% and 1.5% thyme essential oil to chitosan-based film caused decrease in tensile strength of the developed film	Shojaee-Aliabadi et al. [121]
Rosemary essential oil	Addition of 0.5–1.5% rosemary essential oil to an alginate-based film increased the elongation capacity of the film	Hosseini et al. [56]
Thymol and carvacrol essential oil	Addition of 0.5–1.5% of thymol and carvacrol to polypropylene film increased the elongation capacity of the film	Ramos et al. [104]
Nano particles (Unmodified montmorillonite (Na-MMT), Organically modified montmorillonite (Cloisite 30B), Nano-silver, and Ag-zeolite)	Addition of the nanoparticles caused increase in the tensile strength of the developed film due to intercalation of the nanoparticles. However, the Vapour permeability of the film was decreased with the addition of the nanoparticles	Rhim et al. [105]
Silver nano particles and organoclay	Addition of silver nano particles and organoclay (Closite 30 B) increased the transparency, UV barrier, and water vapour barrier properties of the packaging system	Kanmani and Rhim, [67, 68]
Nanoparticles	Incorporation of 2% nanoparticles increased glass transition temperature (Tg) of the chitosan-based polymer film	Dehnad et al. [29]
Articot DLP 02, Artemix cosona, Auranta FV and Sodium octanoate	Addition of articot DLP 02, Artemix cosona, Auranta FV and sodium octanoate as antimicrobial agents resulted in increase in thickness, color and transparency of the packaging system	Clarke et al. [24]
Cinnamon essential oil	Addition of cinnamon essential oil at concentration of 0.5–2% resulted in the increase in extensibility of the film	Ojagh et al. [92]
Zataria multiflora Boiss and Mentha pulegium essential oils	Addition of Zataria multiflora Boiss and Mentha pulegium essential oils caused reduction in the permeability of the Kcarrageenan based packaging films	Shojaee-Aliabadi et al. [121]

storage at 4 °C for 7 days [55]. Zein films carrying lysozyme and di-sodium EDTA showed antimicrobial activity against pathogenic microflora in ground beef patties [130]. Films functionalized with naturally obtained antimicrobials have therefore served immensely in promoting quality and safety of food products.

Edible Coatings

Edible films are majorly convoluted from biopolymers, contributing significantly in maintaining safety and stability of food products. Structurally, the edible coatings are thin films placed on the food surface and act as a barrier against surroundings thereby forming a microenvironment around the surface of application [107]. Edible coatings bearing antimicrobials sustain their antimicrobial activity by controlled diffusion rate retaining their concentrations that is sufficient to produce viable antimicrobial effect. Coating with these edible films is generally done either by dipping the food product in the coating solution or by spraying the coating material on the products either by electrodeposition or my conventional brushing. The successful use of these coating for the preservation of food quality has been reported by several studies, Aloui and Khwaldia Aloui and Khwaldia [2] have given a descriptive review about the use of these edible coatings.

Shelf-life of fresh-cut apple coated with apple puree-alginate coating was prolonged as a result of microbial inhibition by lemongrass and oregano oil. Lemongrass oil at 1.5% and oregano oil at 0.5% showed enormous antimicrobial activity against *L. innocua* reducing its populations by at least 4 log cycles, along with inhibition of general microflora like yeasts molds and psychrophiles [52]. A similar coating composed of alginate incorporated with lemongrass essential oil reduced the counts of microflora in fresh-cut pineapple, thereby extending its shelf-life [10], similar results have been reported by Han et al. [50]. Edible coatings constituted by whey protein isolate and oregano essential oil increased the shelf-stability of chicken breasts stored under refrigeration from 6 to 13 days maintaining microbial counts below acceptable limits [96]. Chitosan coatings containing lactoperoxidase well maintained trout fillets at 4 °C for 16 days

[63], and when enriched with garlic oil improved the keeping quality of shrimp meat [6]. Similarly, many other studies report the antimicrobial efficacy of essential oils derived from *Pimpinella affinis* in silver fillets [5], horsemint in bighead carp fillets [54], and oregano in rainbow trout [57]. The antimicrobial effects of chitosan were synergized by the addition of allyl isothiocyanate (AITC), applied together as an edible coating on cantaloupe. *Salmonella* inoculated onto cantaloupe reduced drastically due to the applied edible coating. Moreover, the quality parameters were not affected throughout the shelf-life of cantaloupe [21]. Antimicrobial effect of carvacrol was extended to preserve minimally processed pumpkin through the application of cassava starch based edible coating. Survival of inoculated bacteria onto pumpkin was monitored along with the natural microflora. At the minimum inhibitory concentration of carvacrol, the counts of *E. coli*, *S. Typhimurium*, *A. hydrophila*, *S. aureus*, coliforms and psychrotrophs were tremendously reduced on the seventh day of storage [113]. Guerreiro et al. [45] have reported the effect of cinnamon leaf oil integrated in edible pectin coatings, and established that there is a complete inhibition of fungal decay caused due to *Botrytis cinerea* for 15 days storage of table grapes stored under 10 °C. Besides, microbial quality of strawberries stored at 0.5 °C for 14 days was reviewed by observing yeast and mould count and population of aerobic mesophiles. The results established antimicrobial effectiveness of citral and eugenol carried by alginate and pectin coatings applied to the strawberries Trout fish fillets coated with gelatin functionalized with cinnamon oil were preserved against microbial spoilage without any compromise on quality during storage [3].

Green tea extracts contained in tapioca starch-based coatings exhibited pronounced antagonism for aerobes, yeast and molds in fruit salads, and Gram-positive bacteria in romaine hearts and pork slices. Modified chitosan carrying limonene and peppermint was applied as antimicrobial coating over strawberries. Limonene preserved the quality attributes of strawberries better and extended their shelf-life. An antimicrobial formulation comprising of lactic acid, citrus extract, and lemongrass EO was applied on cauliflower florets mediated by an edible coating. *Listeria innocua* inoculated onto the florets was completely inhibited after storage at 4 °C for seven days because of the coating [14]. The application of trans-cinnamaldehyde arbitrated via composite-coating system that exhibited significant regarding overall quality preservation of melons and watermelons stored at 4 °C for 15 days [87], 123.

Over the recent years, application of nano-emulsions as edible coatings carrying essential oils has increased. Nano-emulsions consist of oil droplets in nanometers range dispersed in an aqueous phase which is continuous, and the droplets being surrounded by an emulsifier (Fig. 3). The success of this mode of application could be deciphered from the fact that such coatings have proven to be propitious in terms of improving the safety and stability of fresh produce [2]. One such system was designed by Kim et al. [74], wherein carnauba wax acted as the dispersion medium for lemongrass essential oil forming a nano-emulsion to be applied as an edible coating on grape berries. The quality in terms of texture, and nutritive value was found to be intact in addition to combating the growth of *E. coli* and *Salmonella* during storage. Salvia-Trujillo et al. [111] inferred similar results for fresh-cut Fuji apples for 15 days storage on treating with lemongrass EO carried as nano-emulsion in sodium-alginate based coatings. Citrus essential oil-chitosan nano-emulsion when applied to silver pomfret

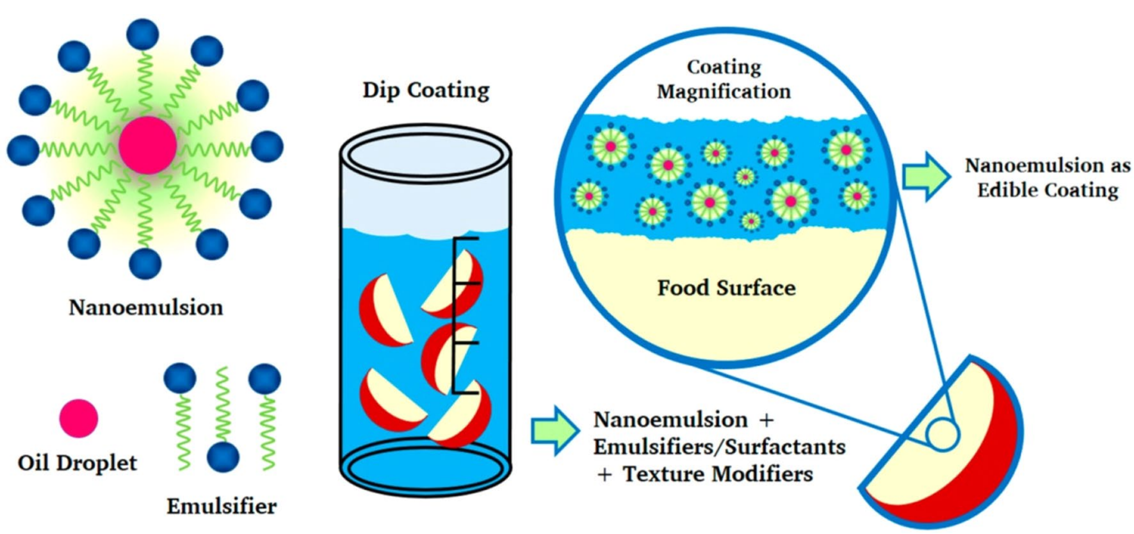

Fig. 3 Nano-emulsion in edible coatings, food interaction [137]

arrested the microbial growth and brought an enhancement in its shelf-life from 12 to 16 days [134]. A nano-emulsion of alginate-basil oil was investigated for its ability to preserve okra against fungal attack and desiccation. Okra pods coated with nano-emulsion (2000 ppm) followed by inoculation with *Penicillium chrysogenum* and *Aspergillus flavus* spores exhibited complete aversion of rotting by the end of storage period [46].

Nisin incorporated through edible coatings of galactomannans and sodium-caseinate retarded the growth of *L. monocytogenes* in Ricotta cheese thereby conferring an extension in its shelf-life [81]. Natamycin, in kashar cheese applied as part of an edible coating system inhibited the growth of *A. niger* and *Penicillium roquefortii* [129]. Semi-hard cheese coated with natamycin also presented growth retardation of yeasts and moulds throughout the storage period [39]. Nisaplin and Guardian applied through an edible coating system of gelatin suppressed *L. monocytogenes* on Turkey-bologna stored at 4 °C [86]. Similar inhibition was reported in ricotta cheese [81] and fresh beef by application of nisin through edible coatings. Natamycin at 1% incorporated in chitosan was found effective in reducing microbial counts on strawberry stored at 4 °C for 40 days [33]. Application of an edible coating of alginate incorporated with pediocin effectively reduced microbial counts by about 4 log cycles in minimally processed papaya stored at 4 °C for 21 days [90]. In another investigation conducted by Cisse et al. [23], lactoperoxidase applied through chitosan coatings was seen to reduce fungal proliferation of mangoes. In hard-boiled eggs, chitosan-lysozyme coatings effectively brought a fourfold reduction in the counts of *S. enteritidis* throughout the storage period of 4 weeks at 10 °C [120]. All these reports elucidate the success of edible coatings as delivery agents for natural antimicrobials, both in terms of preserving their activity and assisting in food preservation as a natural outcome.

Antimicrobial Sachets

Antimicrobial sachets are formed by placing the active antimicrobial compounds inside the carrier located inside the sachet, followed by its sealing. These sachets are also termed as emitters because of their selective permeability for the antimicrobials and capacity to release the active compounds slowly into the food products in which they are placed. Emission of the active components depends on their volatility, compatibility with the carrier, constituting compounds, permeation time, volume of packaging, and the conditions to which the sachets are exposed. Release of active compounds from the sachets is mainly triggered by optimum relative humidity and temperature inside the package headspace diffusing according to the concentration gradient created across the sachet [94]. This section summarizes the applicability of antimicrobial sachets in preserving various food products.

In a study done by Villa-Rodriguez (2015), 100 g of sliced tomatoes were placed over polystyrene trays, along with antimicrobial sachets prepared by incorporating garlic essential oil –β–cyclodextrin microcapsules in cellulose tea sachets (Fig. 4). It was established that 1 g of the composite microcapsules was sufficient for prevention of microbial proliferation of the cut tomatoes during storage period. On similar lines, antifungal sachets comprising lemongrass and oregano EO were used as part of packaging system for mangoes. Lemongrass EO-sachets pronouncedly decreased the aerobic mesophiles, yeast, and mold counts during the storage period. Sachets containing bearing thyme essential oil

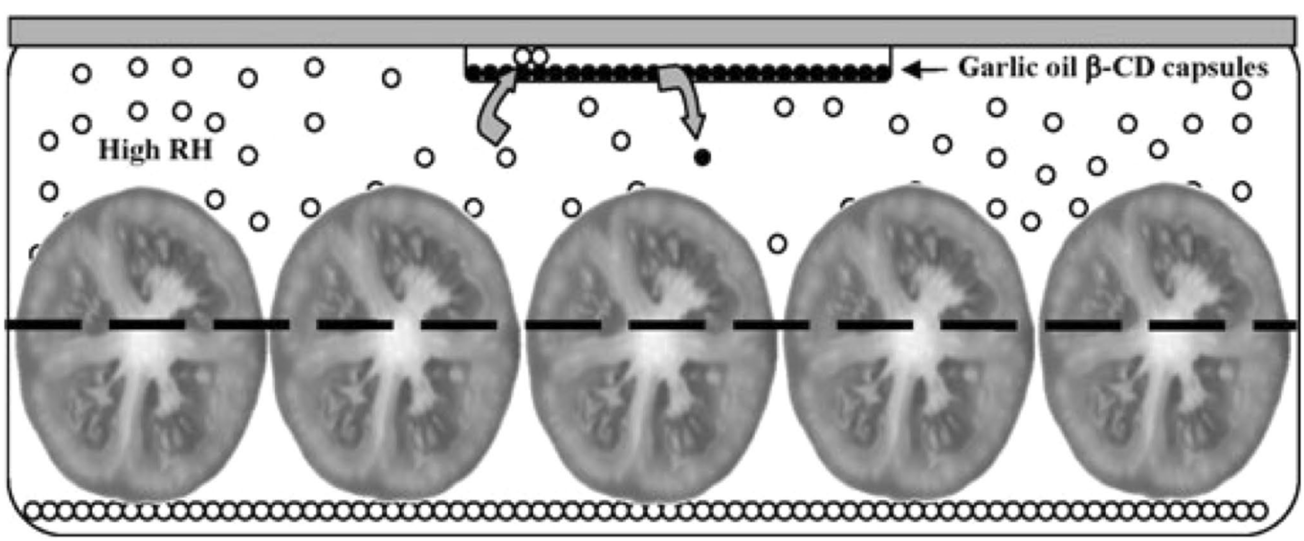

Fig. 4 Encapsulated EO capsules inside a sachet contained in polystyrene trays [9]

and a mix of thyme and rosemary essential oils exhibited a marked anti-listerial activity, along with reduction of lactics and total aerobes in shredded mozzarella cheese under cold storage [51]. In an investigation carried by Espitia et al. [38], cinnamon EO has been reported to have potential antimicrobial characteristics [4], therefore cinnamon EO-containing sachets were found to inhibit the development of postharvest diseases in papaya thereby retaining the quality of the product, Oregano EO based sachets were found to minimize growth of fungus on bread surface [97]. AITC sachets applied to cottage cheese inhibited the growth of yeasts and molds significantly. In mozzarella cheese, Pereira et al. [98] reported a 4-log reduction of *Staphylococcus* because of AITC sachets. A similar antimicrobial sachet was reported by Seo et al. [115], in which AITC was entrapped in calcium alginate beads contained in a low-density polyethylene material. The sachet was monitored for its effects against *E. coli* O157:H7 and yeast and molds on spinach leaves. A considerable reduction in microbial counts was seen at the storage temperatures of 4 and 25 °C for 5 days. In addition to what has been stated so far, AITC sachets could even demonstrate antagonistic effect against post-harvest sporulation caused by A. *flavus* in peanuts. Even after 60 days of storage, a five-fold reduction in the population of the microorganism could be seen, implying the safe consumption of peanuts [93]. Figure 5 depicts the AITC antimicrobial sachet inside a packet containing peanuts. Cindi et al. [22] carried out a study centering on the antimicrobial activity of thyme oil (TO) sachets placed in polyethylene terephthalate (PET) punnets containing peaches. TO sachets remarkably reduced the severity of brown rot attributed to *Monilinialaxa* inoculated artificially as well as in that of naturally infected fruits. Sachets constituted of entrapped cinnamon, lemongrass, and oregano EO inside polymeric resin/nonwoven fabric and were applied as antimicrobial systems for papaya. The sachets exhibited significant activity against phytopathogenic fungi *Rhizopus stolonifer, Alternaria alternata*, *Lasiodiplodiatheobromae*, and *Fusarium semitectum* prior to their incorporation in the packaging of papaya. A considerable reduction in the numbers of mesophiles and yeasts and molds was observed in papaya by the end of storage period [38]. Fresh-cut iceberg lettuce was artificially inoculated with *Dickeyachrysanthemi,* a Gram-negative bacillus implicated in causing soft rot in cut fruit and vegetables. Lettuce stored at 20 °C for 5 days in polypropylene containers was treated with oregano EO microcapsules sachets, vapors released from the sachets exhibited significant inhibitory effects against the infected microorganisms as well as the existing microflora [19]. Sangsuwan et al. [112] designed an antimicrobial sachet by placing chitosan beads entrapping lavender and red thyme EO in a filter paper. The sachet was further placed in a clamshell containing strawberries infected with *B. cinerea*. Inhibitory action of the sachet is deducible form the fact that on the 10th day of storage, the decay percentage was minimum in strawberries having access to the EO vapors released from the sachets, as opposed to control showing highest decay index. Antimicrobial sachets have adopted a significant position in active packaging and have demonstrated tremendous benefits with almost all food products tested so far.

Fig. 5 AITC sachet used for peanut packaging [93]

Encapsulated Antimicrobials

Films and coatings although being used enormously, face serious fallouts due to constant desorption leading to loss of activity. To address this issue, encapsulated antimicrobial systems came into being. These antimicrobial systems are formed by placing the active compounds inside the supports like biopolymeric capsules or beads. These microencapsulated antimicrobials can overcome the uncontrolled desorption from the films thereby improving the overall antimicrobial efficiency, that can be ascribed to substantially high area to volume ratio [2]. Anti-microbial microparticles prepared by encapsulation of allyl isothiocyanate (AITC) in gum arabica and chitosan, when added to Kimchi at different concentrations exhibited promising results with shelf-life of the product. Increasing the concentration of AITC considerably suppressed the populations of *Lactobacillus* and *Leuconostoc* species [75]. AITC was also entrapped in beta cyclodextrin and the complex was used as an antimicrobial in fresh cut onions stored at 5 °C for 20 days. The complex not only arrested the growth of aerobic microflora, but also brought a decline in *Listeria* counts during storage [100]. Thymol encapsulated in sodium caseinate was effective in inhibiting food-borne pathogens in milk [96]. Nisin encapsulated in liposomes on addition to milk brought about a lengthening effect in the lag phase of *L. monocytogenes* Scott A at a storage temperature of 8 and 30 °C

[114]. Chitosan nanoparticles loaded with nisin were found to be effective for preservation of cheese [139].

Thymol as a nano-dispersed system exhibited even better results as compared to the conventional application. Thymol applied as a nano-complex of whey protein isolate and maltodextrin, inhibited food-borne pathogens in reduced-fat milk and apple cider [118]. A tremendous anti-listerial activity was reported in milk by the application of nano-emulsified thymol, so much so that the population of bacteria was negligible after 48 h of application [136]. A co-encapsulated system comprising eugenol and thymol in zein/casein nanoparticles was produced by spray drying. On rehydration and application to milk whey, the antimicrobial nanoparticle system proved to be bacteriostatic against *E. coli* and *L. monocytogenes* Scott A [20]. D-Limonene and terpenes extracted from *Melaleuca alternifolia* were encapsulated in a nano-emulsion delivery system and were then tested for their effectiveness on *Saccharomyces cerevisiae*, *E. coli* and *L. delbrueckii*. The encapsulated compounds were effective bacteriostatic and bactericidal agents against the tested microbes. Nano-emulsion encapsulating the antimicrobial components was further found to inactivate *L. delbrueckii* in orange and pear juice [32]. Oregano oil nano-emulsions effectively reduced foodborne bacteria in lettuce stored at 4 °C for 72 h, even at a minimal concentration of 0.1% [13]. On the same line, Jo et al. [64] reported a successful inhibition of *S. aureus* and *Salmonella Typhimurium* in watermelon juice held at 37 °C for 72 h. Sunflower nano-emulsions containing *Zataria multiflora* essential oil prevented lipid oxidation in trout fillets and therefore enhanced their shelf-life [117]. Nanoparticles containing phenolic compounds proved to be more effective than their direct application against pathogenic microflora on meat systems. Chitosan-thymol nanoparticles were applied internally as a coating on PET clamshells surface storing blueberries and cherry tomatoes at 7 °C for 10 days. The nanoparticles demonstrated in vitro antimicrobial activity against *S. aureus, S. typhimurium*, and *L. innocua*, and were also effective in reducing the weight loss of the products [84].

Challenges and Limitations in Use of Natural Antimicrobials in Food Industry

Studies have shown an extensive and promising effect of natural antimicrobial agents owing to increased consumer demand of chemical free food products, that compels the food industries to adopt the natural compounds [102]. However, various challenges regarding technological aspects, sensory quality of foods, food safety and regulatory issues are yet to be tackled [106]. Furthermore, usage and development cost are also unstandardized [27]. The major challenge regarding sensory aspects of food products includes changes in flavor of the food products using plant derived natural antimicrobial agents like essential oils and phenolic extracts, however, incorporation of essential oils in packaging systems can be used as an effective alternative to avoid sensorial degradation of food products [106].

Management of risks associated with the quantity of antimicrobial agents to be used in food products is also one of the major concerns. Allergies related to biological compounds and dose related toxicity is also a major issue that limits the use of these bioactive compounds [106]. Chitosan, a natural and biodegradable antimicrobial used in food industry is extracted from shrimps and shell fishes poses allergy threats to people who are pregnant, lactating, and allergic to shell fishes [30]. Besides, in majority of countries, a limited quantity of various antimicrobials like lactoferin, pediocins, nisin etc. are allowed [102], 122, that result in limited usage of these compounds.

Conclusions and Future Scope

Growing consumer demand for the food products that are free from synthetic preservatives, it became imperative to explore and identify the unconventional yet safe and effective approaches for food preservation and control of the pathogenic outbreaks. A shift to natural antimicrobial agents is also vital to address the resistance of microbes to the antibiotics/synthetic antimicrobials. A wide range of natural antimicrobials are being used either individually or in combination with other agents or techniques to create a multi hurdle effect for microbial growth and survival, thereby improving the safe and extended shelf life of food products. However, there are several other possibilities regarding the exploration of these natural antimicrobials for preservation as well as the improvement of overall quality of the food products [59, 128].

Despite the growing demand of the natural antimicrobials, there are certain limitations like objectionable flavors, authoritarian issues, undesirable interaction with the food components, and non-optimized standards associated with them. Pertaining to the pivotal role of these antimicrobial in food products, further investigation regarding exploration of different techniques, methodologies and optimum dosages for improved extraction and application of these antimicrobials to the food systems without any aversive reason regarding different organoleptic qualities of food products is required.

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Declarations

Conflict of Interest The authors express no conflict of interest with respect to the present review article.

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