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Nanoemulsion-Based Technologies for Delivering Natural Plant-Based Antimicrobials in Foods

David Julian McClements[1]*, *Arun K. Das*[2]*, *Pubali Dhar*[3,4], *Pramod Kumar Nanda*[2] and *Niloy Chatterjee*[3,4]

[1] Department of Food Science, University of Massachusetts, Amherst, MA, United States, [2] Eastern Regional Station, ICAR-Indian Veterinary Research Institute, Kolkata, India, [3] Laboratory of Food Science and Technology, Food and Nutrition Division, University of Calcutta, Kolkata, India, [4] Center for Research in Nanoscience & Nanotechnology, University of Calcutta, Kolkata, India

There is increasing interest in the use of natural preservatives (rather than synthetic ones) for maintaining the quality and safety of foods due to their perceived environmental and health benefits. In particular, plant-based antimicrobials are being employed to protect against microbial spoilage, thereby improving food safety, quality, and shelf-life. However, many natural antimicrobials cannot be utilized in their free form due to their chemical instability, poor dispersibility in food matrices, or unacceptable flavor profiles. For these reasons, encapsulation technologies, such as nanoemulsions, are being developed to overcome these hurdles. Indeed, encapsulation of plant-based preservatives can improve their handling and ease of use, as well as enhance their potency. This review highlights the various kinds of plant-based preservatives that are available for use in food applications. It then describes the methods available for forming nanoemulsions and shows how they can be used to encapsulate and deliver plant-based preservatives. Finally, potential applications of nano-emulsified plant-based preservatives for improving food quality and safety are demonstrated in the meat, fish, dairy, and fresh produce areas.

Keywords: antimicrobials, antioxidants, plant-based, encapsulation, nanoemulsions, nanopesticides, food quality, food safety

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****Correspondence:***
David Julian McClements
mcclemen@umass.edu
Arun K. Das
arun.das@icar.gov.in

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INTRODUCTION

Food preservation technologies increase the safety, quality, and shelf-life of food products, thereby reducing food waste and enhancing the sustainability of the food supply (Pardo and Zufía, 2012). Researchers are therefore continually trying to optimize the efficacy of traditional preservation methods, as well as develop new ones (Bhadekar and Bhola, 2019). Some of the most common preservation approaches involve the thermal processing of foods. Typically, the foods are heated at temperatures ranging from 60 to 100°C for times ranging from a few seconds to a few minutes to inhibit spoilage and pathogenic microorganisms. However, these processes do have some limitations because they can cause undesirable changes in food quality, loss of vital nutrients, involve high energy costs, and utilize valuable resources (Teixeira, 2014). For these reasons, there is growing interest in developing non-thermal preservation methods to overcome these problems (Barba et al., 2018). In particular, there has been an interest in utilizing natural preservatives isolated from animal, plant, or microbial sources because of consumer demand for a more sustainable and healthy food supply (Sharif et al., 2017; Das et al., 2020b). Nevertheless, these ingredients must be developed taking into account economic factors, regulatory restrictions, toxicity problems, customer concerns, etc. (Davidson et al., 2014).

A wide variety of natural substances have been examined for their potential utilization as preservatives in foods, including phytochemicals derived from various botanical sources (Bouarab Chibane et al., 2019; Das et al., 2020b, 2021b), as well as essential oils, such as those isolated from clove (*Syzygium aromaticum*), eucalyptus (*Eucalyptus globulus*), basil (*Ocimum basilicum*), black pepper (*Piper nigrum*), cannabis (*Cannabis sativa*), citronella (*Cymbopogon nardus*), and rosemary (*Salvia rosmarinus*) plants (Chouhan et al., 2017; Falleh et al., 2020). In general, plants are a rich source of natural antimicrobial agents, and researchers are actively working to identify, characterize, and utilize them (Quinto et al., 2019; Rao et al., 2019; Srivastava et al., 2020). However, many plant-based antimicrobials cannot simply be utilized in their free form due to their chemical instability, poor dispersibility in food matrices, or unacceptable flavor profiles. As a result, encapsulation technologies, such as emulsion-based systems, are being developed and employed to overcome these problems. Emulsions are widely used for this purpose because they can simply and affordably be produced using food-grade ingredients and processing operations. A well-designed delivery system can improve the handling, ease of use, stability, and potency of plant-based preservatives.

In this review, we focus on the utilization of oil-in-water nanoemulsions as delivery systems for natural antimicrobials. We begin by reviewing the properties of different kinds of natural preservatives. We then highlight the formulation, fabrication, and properties of nanoemulsion-based delivery systems. Finally, we provide specific examples of the application of antimicrobial nanoemulsions to foods.

PLANT-BASED ANTIMICROBIALS

A wide range of natural substances with antimicrobial activity have been identified in plant-based edible materials (Goel et al., 2020). Many plants produce secondary metabolites and other substances to protect themselves from invading pathogens, such as microorganisms (bacteria, fungi, and viruses), insects, and herbivores. These plant-derived compounds can be categorized into different groups depending on their botanical source, chemical structure, or mechanism of action. Plant extracts, such as herbs and spices, have already been used for centuries in foods for their antimicrobial activities, as well as for their flavors and fragrances (Fan et al., 2018). Herbs and spices exhibit different antimicrobial activities against different foodborne microorganisms depending on their constituents (Tajkarimi et al., 2010; Savoia, 2012). Recently, there has been interest in isolating natural antimicrobials from waste materials recovered from the food and agricultural industries, such as fruit and vegetable waste products (Varzakas et al., 2016; Quinto et al., 2019). Detailed information about different classes of natural antimicrobials have been given elsewhere (Cowan, 1999; Savoia, 2012; Ud-Daula et al., 2016; Meneguetti et al., 2017; Quinto et al., 2019; Rao et al., 2019; Srivastava et al., 2020). The major groups of antimicrobial phytochemicals are summarized in **Table 1**. In this section, we highlight some of the most important plant-based substances with antimicrobial activity.

Phenols and Polyphenols

Phenols and polyphenols are secondary metabolites that are produced by many plants to defend themselves from microbes, insects, and herbivores. Many phenolics are potent antioxidants that can inhibit oxidative changes (such as lipid, phospholipid, or protein oxidation) due to their metal chelating and free radical scavenging properties (Dai and Mumper, 2010; Das et al., 2021b). The major group of phenols and polyphenols include simple phenols, simple phenolic acids, flavonoids, quinines, tannins, and coumarins (**Figure 1**).

Simple Phenols and Phenolic Acids

Simple phenols and phenolic acids consist of a single substituted phenolic ring, which may have one or more hydroxyl groups attached, such as cinnamic and caffeic acids (Cowan, 1999; Othman et al., 2019). The toxicity of these molecules to microorganisms is related to the site(s) and degree of hydroxylation, with higher inhibitory activity linked to a more oxidized structure (Cowan, 1999). The mode of action of these phenolics has been attributed to inhibition of critical microbial enzymes, possibly due to their reaction with sulfhydryl or other functional groups on the enzyme surfaces (Cowan, 1999; Savoia, 2012; Coppo and Marchese, 2014). Other researchers have suggested that the antibacterial activity of phenolic acids is due to disruption of membrane integrity, which results in leakage of essential intracellular components (Borges et al., 2013), direct alteration of microbial metabolism, and inactivation of essential substrates required for microbial growth (Dietrich and Pour Nikfardjam, 2017). The ability of phenolics to inhibit bacteria by altering their surface properties is shown schematically in **Figure 2**. For example, ferulic acid has been found to decrease the hydrophobicity of *Pseudomonas aeruginosa* and alter bacterial polarity by interfering with surface electron acceptors on both Gram-positive and Gram-negative strains (Borges et al., 2013). Recently, Liu J. et al. (2020) provided a detailed account of the antibacterial effect of various phenolic acids against both food-borne pathogens and human pathogenic strains.

Flavonoids

Flavonoids are a class of natural molecules typically isolated from flowers and fruits, which are reported to exhibit potent antimicrobial and antioxidative activities (Górniak et al., 2019). Flavonoids have a 2-phenyl-benzo-γ-pyrane core comprising of two benzene rings (Rings A and B) linked through a heterocyclic pyran or pyrone (Ring C). Flavonoids can be classified into various groups based on their level of oxidation and unsaturation, such as flavones, isoflavones, flavan-3-ols (catechins), flavanones, and anthocyanidins (Cowan, 1999; Falcone Ferreyra et al., 2012). These secondary plant metabolites are synthesized in response to microbial infection and are reported to have strong antimicrobial action against a wide range of pathogenic microorganisms (Othman et al., 2019). For this reason, they are increasingly being explored for use in the pharmaceutical and healthcare

TABLE 1 | Major classes of antimicrobial compounds, proposed mechanisms of action, and antimicrobial spectrum of plants and plant extracts.

Class	Subclass	Example	Mechanism	Selected antimicrobial spectrum
Phenolics	Simple phenols	Catechol, epicatechin, eugenol	Substrate deprivation, membrane disruption	*Listeria monocytogenes* *Staphylococcus aureus* *Escherichia coli* O157:H7 *Salmonella enterica* *Vibrio cholerae* *Pseudomonas aeruginosa* *Acinetobacter baumannii* *Klebsiella pneumoniae* *Aspergillus flavus* *Penicillium* sp. *Cladosporium* sp.
	Phenolic acids	Cinnamic acid, P-coumaric acid, ferulic acid, caffeic acid, vanillic acid	Enzyme inhibition, membrane disruption, bind to cell wall proteins	
	Anthocyanins	Cyanidin, petunidin	–	
	Flavones Flavonoids	Apigenin, chrysin, rutin, catechin, epicatechin	Complex with cell wall, bind to adhesins	
	Flavanones	Abyssinone, naringenin, fisetin	Inactivate enzymes, inhibit HIV reverse transcriptase	
	Quinones	Anthraquinone, benzoquinone, hypericin, naphthoquinone, plastoquinone, pyrroloquinoline quinone	Bind to adhesins, inactivate enzymes	*S. aureus, P. aeruginosa* *Bacillus subtilis,* *Cryptococcus neoformans*
Tannins	Ellagitannin Condensed tannins	Tannic acid, gallic acid, proanthocyanidins	Bind to cell wall of bacteria and inhibit their growth, bind to proteins, bind to adhesins, inhibit enzyme etc.	*Bacillus cereus, S. aureus, L. monocytogenes, S. enterica, Campylobacter jejuni*
	Coumarins	Ammoresinol, warfarin, ostruthin, anthogenol, agasyllin	Membrane disruption	*Salmonella Typhimurium, Salmonella Enteritidis, Vibrio parahaemolyticus, S. aureus, L. monocytogenes, E. coli* O157:H7
Terpenoids, essential oils	Capsaicin	Carotenoids, terpinene Isopentenyl, pyrophosphate, thymol, geraniol, linalool	Destruct outer and inner membrane, induce leakage, loss of membrane integrity, changes in cytoplasm	*Salmonella Typhi, S. aureus, P. aeruginosa, V. cholerae*
Alkaloids		Berberine, piperine	Intercalate into cell wall and/or DNA	*Proteus mirabilis, E. coli, P. aeruginosa, S. aureus, K. pneumoniae*
Lectins and polypeptides		Mannose-specific agglutinin, fabatin, concanavalin A, wheat germ agglutinin, *Aleuria aurantia* lectin	Block viral fusion or adsorption Form disulfide bridges	*Candida albicans, S. aureus, B. subtilis, E. coli, P. aeruginosa*

Adopted from Cowan (1999), Upadhyay et al. (2014), Zhang et al. (2016), Chouhan et al. (2017), Othman et al. (2019), Quinto et al. (2019), and Tanhaeian et al. (2020).

industries (Górniak et al., 2019). They are proposed to exert their antimicrobial activities by interacting with bacterial cell walls, thereby disrupting the cell membranes and interfering with critical biochemical pathways (Reygaert, 2014). The ability of many flavonoids to generate reactive oxygen species (ROS), such as epicatechin, epigallocatechin gallate, and quercetin, that can damage important cellular processes has also been proposed as a potential mechanism for their antioxidant activity (Fathima and Rao, 2016). Anthocyanins, which are another class of flavonoids, have also been reported to exhibit strong antibacterial activity against food-borne pathogens, such as *Salmonella Enteritidis*, *Vibrio parahaemolyticus*, *Staphylococcus aureus,* and *Listeria monocytogenes*. For instance, in a recent study, anthocyanins were shown to exert their antibacterial activities by damaging microbial cell membranes and interfering with critical biochemical pathways responsible for cell growth and reproduction (Sun et al., 2018). A comprehensive review of the antimicrobial activities of different flavonoids has been published recently (Górniak et al., 2019).

Alkaloids

Alkaloids are a structurally diverse group of secondary metabolites with broad antimicrobial activity that are distinguished by the fact they are heterocyclic nitrogenous compounds (Cushnie et al., 2014; Upadhyay et al., 2014). Based on the chemical structure, they can be divided into

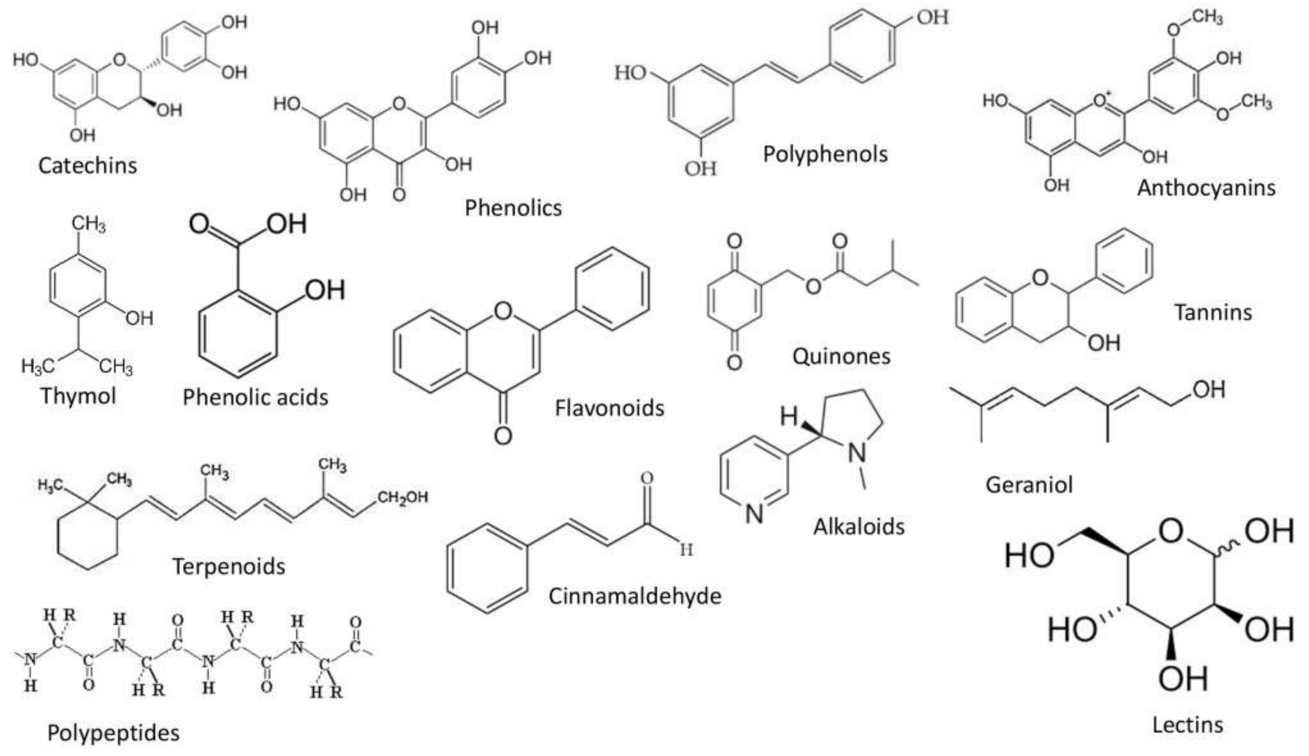

FIGURE 1 | The structure of some phenols and polyphenols that may be incorporated into nanoemulsions.

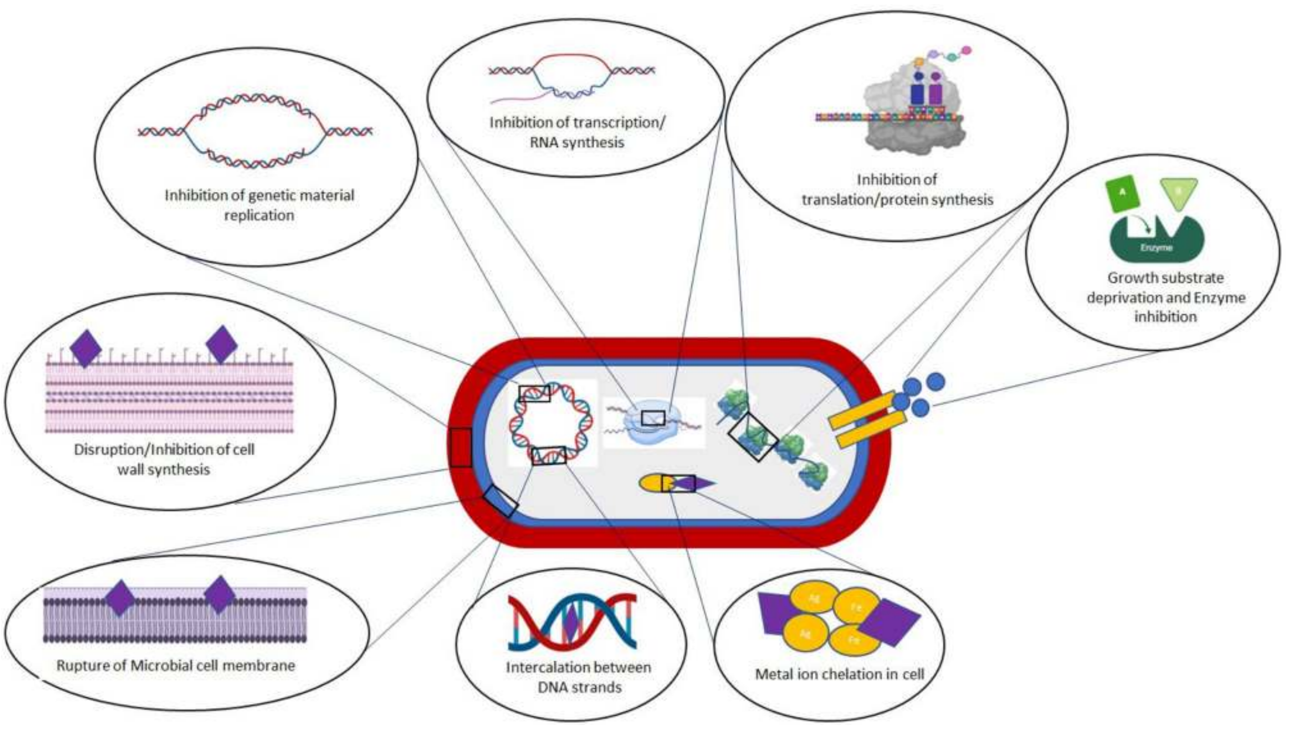

FIGURE 2 | Mechanisms of action of plant-based antimicrobial agents.

proto-alkaloids or atypical alkaloids (like hordenine or *N*-methyltyramine, colchicine) having no heterocyclic compounds and typical alkaloids (such as hygrines belonging to the pyrrole and pyrrolidine group, and quinine belonging to the quinoline group) having heterocyclic compounds (Evans, 2009). Typical alkaloids are subdivided into different classes depending on the nature of the heterocyclic ring structure. The most common compounds in this group are codeine and morphine. Being secondary plant metabolites, alkaloids have been reported to have potent pharmacological activities including bacteriostatic, as well as bactericidal effects. As an example, plant alkaloids (1.67 mg/mL) isolated from *Callistemon citrinus* and *Vernonia adoensis* leaves prevented the growth of *S. aureus* and *P. aeruginosa* (Mabhiza et al., 2016). Indeed, these alkaloids have an efficacy comparable to that of ampicillin, a standard antibiotic. The alkaloids from *C. citrinus* also showed strong antimicrobial activity against *S. aureus* with a MIC of 0.0025 mg/mL and a minimum bactericidal concentration (MBC) of 0.835 mg/mL (Mabhiza et al., 2016). Diterpinoid alkaloids extracted from buttercups and the stem bark of *Clausena anisata* (Wild) were found to have good antibacterial properties (Upadhyay et al., 2014). Alkaloids from *Achyranthes aspera* (Amaranthaceae) plants were tested for their antibacterial activities against four species viz. *Bacillus subtilis*, *Escherichia coli*, *P. aeruginosa,* and *S. aureus* (Mishra, 2018). Alkaloids at a concentration of 40 mg/ml showed the highest bacterial activity against *B. subtilis.* Sanguinarine, a benzophenanthridine alkaloid extracted from the root of *Sanguinaria canadensis* L., was found to possess inhibitory activity against methicillin-resistant *S. aureus* (MRSA) bacteria, which is an organism known for its resistance to almost all antibacterial agents (Obiang-Obounou et al., 2011). The antibacterial properties of plant alkaloids has been attributed to their ability to intercalate into cell wall or DNA within microbial cells (Savoia, 2012) and to inhibit the ATP-dependent transport of compounds across the cell membrane (Mabhiza et al., 2016), thereby leading to impaired cell division and cell death (**Figure 2**).

Tannins

Tannins are a class of water-soluble polyphenolic biomolecules mainly found in the major parts of higher plants, such as leaves, bark, fruits, and roots (herbaceous and woody plants) (Scalbert, 1991; Upadhyay et al., 2014). Traditionally, tannins have been used for tanning leather or precipitating gelatin from solution (Cowan, 1999). They are mainly categorized into two categories: hydrolyzable tannins (HTs) and condensed tannins (CTs). In the case of HTs like gallotannin, hydroxyl groups of a carbohydrate moiety are esterified with gallic acid or ellagic acid, whereas CTs, which are also commonly called proanthocyanidins (PAs) like catechin, are mainly composed of oligomers of the flavan-3-ols and other related flavanol residues mainly linked by carbon-carbon bonds and yield anthocyanidins on acid digestion (Akiyama et al., 2001). Catechins obtained from tea leaves are comprised mainly of epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate (Scalbert, 1991; Hamilton-Miller, 1995). Tannins or tannin-rich plant extract have been reported to have high bacteriostatic or bactericidal effects against both Gram-positive and Gram-negative bacteria, such as *S. aureus, P. aeruginosa, E. coli*, *Yersinia enterocolitica, Bacillus cereus, and Enterococcus faecalis* (Pandey and Negi, 2018; Dabbaghi et al., 2019; Belhaoues et al., 2020). Major applications of tannins are in the leather, food, biomedical, and health care industries (Upadhyay et al., 2014; Kaczmarek, 2020). The antimicrobial activity of tannins is mainly due to inhibition of nutrient uptake by bacteria and interference with membrane transport proteins (Scalbert, 1991; Akiyama et al., 2001; Belhaoues et al., 2020). However, this activity depends on many factors, including the tannin concentration and type, microbial species, pH, temperature, and food matrix effects (Kaczmarek, 2020).

Coumarins

Coumarins are a class of phenolic aromatic benzopyrones compounds that have fused benzene and alpha pyrone rings (Kennedy and Thornes, 1997). There are more than 1,300 coumarins identified and many of these exhibit antimicrobial activity (Upadhyay et al., 2014). Coumarins, such as aegelinol and agasyllin extracted from the roots of *Ferulago campestris* have been shown to exhibit strong antibacterial activity against *S. aureus, Salmonella Typhi, Enterobacter cloacae,* and *Enterobacter aerogenes* (Basile et al., 2009). The minimum inhibitory concentration MIC for aegelinol was 16 μg/mL whereas it was 32 μg/mL for agasyllin. Again, various pyrenylated coumarin compounds from dichloromethane extract of *Prangos hulusii* roots exhibited antimicrobial activity against *B. subtilis* and *Klebsiella pneumoniae* at a concentration between 5 and 125 μg/mL (Tan et al., 2017). In a recent study, coumarins, imperatorin, and chalepin (at a concentration of 20 μg/mL) isolated from the root extract of plant *C. anisata* exhibited strong antibacterial activity against *S. aureus* and *B. subtilis* (Tamene and Endale, 2019).

Terpenoids and Essential Oils

Essential oils (EO) are an important source of plant-based preservatives that exhibit antimicrobial activity against various spoilage organisms and foodborne pathogens. EOs are extracted from various parts of plants, including leaves, flowers, seeds, barks, bulbs, buds, fruits, roots, and rhizomes (Chouhan et al., 2017). They often impart a characteristic aroma and taste to foods because of their unique composition. Commercially, steam distillation is the most frequently used method for the isolation of essential oils. Essential oils typically contain a complex mixture of numerous (20–80) volatile substances, including terpenes, terpenoids, phenols, and non-terpene compounds (Rao et al., 2019). Various factors impact the composition of essential oils, including geographical area, habitat, harvest time, storage conditions, and extraction methodology (Ud-Daula et al., 2016). For example, thyme oil extracted from *Thymus vulgaris* has six chemotypes based on the dominant component present, like thymol, carvacrol, linalool, geraniol, γ-terpineol, and complex trans-thujan-4-ol/terpinen-4-ol (Rao et al., 2019).

The antibacterial activity of essential oils depends on their composition (Kordali et al., 2005; Hussain et al., 2008). For example, oregano oils exhibited greater antimicrobial activity against *L. monocytogenes*, *B. cereus, E. coli,* and *P. aeruginosa* when mixed with thyme oil than with pure oils (Gutierrez et al., 2008). Recently, in another study, the combination of clove (40%), basil (20%), and thyme (40%) oil was reported to have a synergistic effect on the inhibition of yeasts and molds (Sharma et al., 2020). This synergistic effect not only improves their antimicrobial efficiency but also reduces the doses required, which can reduce costs, toxicity, and off-flavor effects. Numerous researchers have proposed several mechanisms of action for the antimicrobial activity of essential oils on microbes, such as damaging cell membranes, decreasing the proton-motive force, or interfering with key biochemical pathways (Di Pasqua et al., 2006; Swamy et al., 2016; Quinto et al., 2019; Rao et al., 2019; Tanhaeian et al., 2020). Numerous studies have shown that essential oils can be used as effective antimicrobial agents in the pharmaceutical and food industries (Diao et al., 2014; Chouhan et al., 2017; Rao et al., 2019; Tanhaeian et al., 2020). The MIC values of numerous types of essential oils against different pathogenic microorganisms are shown in **Table 2**.

Lectins and Polypeptides

Many plants produce antimicrobial peptides (AMPs) in response to external stresses and infections. Plant-derived AMPs are typically relatively small ($<$10 kDa), cysteine-rich, cationic, and amphipathic molecules (Meneguetti et al., 2017), such as thionin, lunatusin, defensin, heveins, knottin, 2S albumin-like proteins, α-hairpinin, hispidulin, lipid transfer protein, thionein: alpha-1-purothionin, puroindolines, cyclotides (López-Meza et al., 2015; Tam et al., 2015; Meneguetti et al., 2017). These peptides exhibit potent antimicrobial activity toward a broad spectrum of microorganisms including bacteria, viruses, fungi, and protozoa (Franco, 2011). For example, lunatusin (7 kDa) extracted from Chinese lima bean (*Phaseolus lunatus* L.) exhibits antibacterial toward *Bacillus megaterium*, *B. subtilis*, *Proteus vulgaris*, and *Mycobacterium phlei*, as well as antifungal activity toward *Fusarium oxysporum*, *Mycosphaerella arachidicola*, and *Botrytis cinerea* (Wong and Ng, 2005). Hispidulin (5.7 kDa) extracted from the seeds of *Benincasa hispida* exhibits strong antimicrobial and antifungal activities against various pathogens (Sharma et al., 2014). The most widely accepted mechanism of action of AMPs is their ability to interact with lipid components in microbial cell surfaces, resulting in membrane disruption (Yeaman and Yount, 2003; Barbosa Pelegrini et al., 2011). These cationic peptides may also exhibit antimicrobial activity through their electrostatic interactions with anionic microbial cell membranes (Barbosa Pelegrini et al., 2011). Three main mechanism of pore formation in bacterial cell membranes have been identified: transmembrane pore formation; membrane solubilization/disruption; and wormhole formation (Matsuzaki et al., 1995; Tam et al., 2015; Samriti and Biswas, 2018; Mercer et al., 2020).

NANOENCAPSULATION OF PRESERVATIVES

An effective antimicrobial compound should interact with the intended target but only have minimal interactions with non-specific targets so as to increase its potency and reduce its deleterious effects (Mothershaw and Jaffer, 2004). The efficacy of natural antimicrobials can be altered by food matrix effects, such as phase partitioning or ingredients interactions, thereby reducing their interactions with microbes (Weiss et al., 2015). Numerous researchers have tried to overcome these effects by encapsulating the antimicrobials in edible colloidal delivery systems (Acevedo-Fani et al., 2017b; Mousavi Khaneghah et al., 2018).

Encapsulation involves trapping the antimicrobials within small particles (Abd El Kader and Abu Hashish, 2020). These particles can be designed to increase the water-dispersibility, resistance to environmental conditions, and potency of antimicrobials (Ray et al., 2016; Ferreira and Nunes, 2019). Moreover, they can be designed to mask undesirable flavors or to control the release profile of the antimicrobials (Baranwal et al., 2018; Vitiello et al., 2018). Nanotechnology is increasingly being utilized to design effective antimicrobial delivery systems by manipulating their structures on the nanoscale (Aruguete et al., 2013; Gupta et al., 2019). In general, the encapsulation and release properties of nano-enabled delivery systems depends on their composition and structure, which are governed by the ingredients and processing methods used to fabricate them (Martins et al., 2015; Akhavan et al., 2018; Taheri and Jafari, 2019). Oil-in-water nanoemulsions, which contain lipid nanoparticles dispersed in water, are currently the most commonly used nano-enabled delivery system for natural antimicrobials, because they can easily be manufactured from food-grade ingredients using common processing methods, such as mixing and homogenization (Tahmasebi et al., 2016; Lohith Kumar and Sarkar, 2018).

Different classes of antimicrobial nanoemulsions can be developed based on the type and location of the antimicrobials they contain (**Figure 3**). First, the oil phase itself may be inherently antimicrobial (like an essential oil). Second, a lipophilic antimicrobial (like a polyphenol) may be dissolved within an inert oil phase (like corn oil). Third, an antimicrobial emulsifier may be used to form and stabilize the nanoemulsions. Fourth, a nanoemulsion may contain a combination of different antimicrobials in different locations, which can lead to strong potency. The antimicrobial agents may move from the nanoparticles to the microbe surfaces through various mechanisms, including simple molecular diffusion, micellar diffusion, or collisions.

NANOEMULSION CHARACTERISTICS

Nanoemulsions are colloidal dispersions consisting of two immiscible fluids, with one of them being dispersed as small particles ($d < 200$ nm) in the other one (Salvia-Trujillo et al., 2016). These systems are thermodynamically unstable, but can

TABLE 2 | Minimum inhibitory concentration (MIC) of essential oils against various microorganisms.

Essential oil	Plant source	MIC values	Target microorganisms	References
Cinnamon	*Cinnamomum zeylanicum*	8 mg/mL 2 mg/mL 8 mg/mL 4 mg/mL 0.5 mg/mL 1 mg/mL	*Acinetobacter* *Klebsiella pneumoniae* *Proteus vulgaris* *Enterococcus faecalis* *Staphylococcus aureus* *Staphylococcus epidermidis*	Aumeeruddy-Elalfi et al., 2015
Clove basil	*Ocimum gratissimum*	6 μg/mL ≥24 μg/mL 0.75 μg/mL	*Escherichia coli* *Pseudomonas aeruginosa* *S. aureus*	Hyldgaard et al., 2012
Lavender	*Lavandula officinalis*	2,000 ppm 1,000–1,200 ppm	*E. coli* *S. aureus*	Martucci et al., 2015
Oleoresin	*Liquidambar formosana*	156 μg/mL 156 μg/mL 156 μg/mL 78 μg/mL 156 μg/mL 313 μg/mL 625 μg/mL	*Bacillus cereus* *Cutibacterium acnes* *S. aureus* *S. epidermidis* *Streptococcus pyogenes* *P. aeruginosa* *Serratia marcescens*	Decarlo et al., 2020
Lemon bergamot	*Citrus limon* *Citrus bergamia*	4% (v/v)	*S. aureus, E. coli* *Aspergillus niger*	Çakmak et al., 2020
Frankincense Myrtle Thyme Lemon Oregano Lavender	*Boswellia sacra* *Myrtus communis* *Thymus vulgaris* *C. limon* *Origanum vulgare* *Lavandula officinalis*	0.1% to >50% (v/v)	*S. aureus*, *E. faecalis*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*	Man et al., 2019
Betel leaf	*Piper betle*	8 mg/mL 4 mg/mL 4 mg/mL 4 mg/mL 0.5 mg/mL 0.5 mg/mL	*Acinetobacter* *K. pneumoniae* *P. vulgaris* *E. faecalis* *S. aureus* *S. epidermidis*	Aumeeruddy-Elalfi et al., 2015
Turmeric	*Curcuma longa*	>200 ppm >200 ppm 200 ppm 50 ppm 100 ppm 100 ppm	*E. coli* *P. aeruginosa* *B. cereus* *Bacillus coagulans* *B. subtilis* *S. aureus*	Negi et al., 1999
Willow herb	*Epilobium parviflorum*	10–40 μg/mL	*E. faecalis*, *S. aureus*, *E. coli*, *P. aeruginosa*	Bajer et al., 2017
Lemongrass	*Cymbopogon citratus*	0.6 μL/mL 2.5 μL/mL 0.6 μL/mL	*E. coli* *Salmonella Typhimurium* *S. aureus*	Burt, 2004
Sweet marjoram	*Origanum majorana*	45.0 mg/mL 22.5 mg/mL 45.0 mg/mL 22.5 mg/mL 22.5 mg/mL	*E. coli* *Salmonella enterica* *P. aeruginosa* *B. subtilis* *S aureus*	Moumni et al., 2020
Tea bush	*Lippia* spp.	2.5–>80 μl/mL 5.0–>80 μl/mL 0.6–40 μl/mL 5–10 μl/mL	*E. coli* *Shigella dysenteriae* *S. aureus* *B. cereus*	Bassole et al., 2003
Lantanas	*Lantana xenica*	9.1 mg/mL 9.1 mg/mL	*Proteus mirabilis* *B. cereus*	Juliani et al., 2002
Rosemary Peppermint Anise	*Rosmarinus officinalis* *Mentha piperita* *Pimpinella anisum*	10 mg/mL 10 mg/mL 10 mg/mL	*Clostridium perfringens*	Radaelli et al., 2016
Lemon myrtle	*Backhousia citriodora*	200 μg/ml 50 μg/ml	*E. coli* *S. aureus*	Thielmann et al., 2019

(Continued)

TABLE 2 | Continued

Essential oil	Plant source	MIC values	Target microorganisms	References
Origanum Thyme Tea tree Basil Chamomile	*Thymus capitatus* *T. vulgaris* *Melaleuca alternifolia* *Ocimum basilicum* Matricaria chamomilla	0.06–0.5% (v/v) 0.06–1% (v/v) 0.12–1% (v/v) 0.25–4% (v/v) 2–>4% (v/v)	Methicillin-resistant *S. aureus*	Sakkas et al., 2018
Ravintsara	*Cinnamomum camphora*	6,400 µg/ml 3,200 µg/ml	*E. coli* *S. aureus*	Thielmann et al., 2019
Origanum Thyme Tea tree Basil Chamomile	*T. capitatus* *T. vulgaris* *M. alternifolia* *O. basilicum* *M. chamomilla*	0.25–1% (v/v) 0.5–2% (v/v) 1–4% (v/v) 4–>4% (v/v) >4% (v/v)	Vancomycin-resistant Enterococci	Sakkas et al., 2018

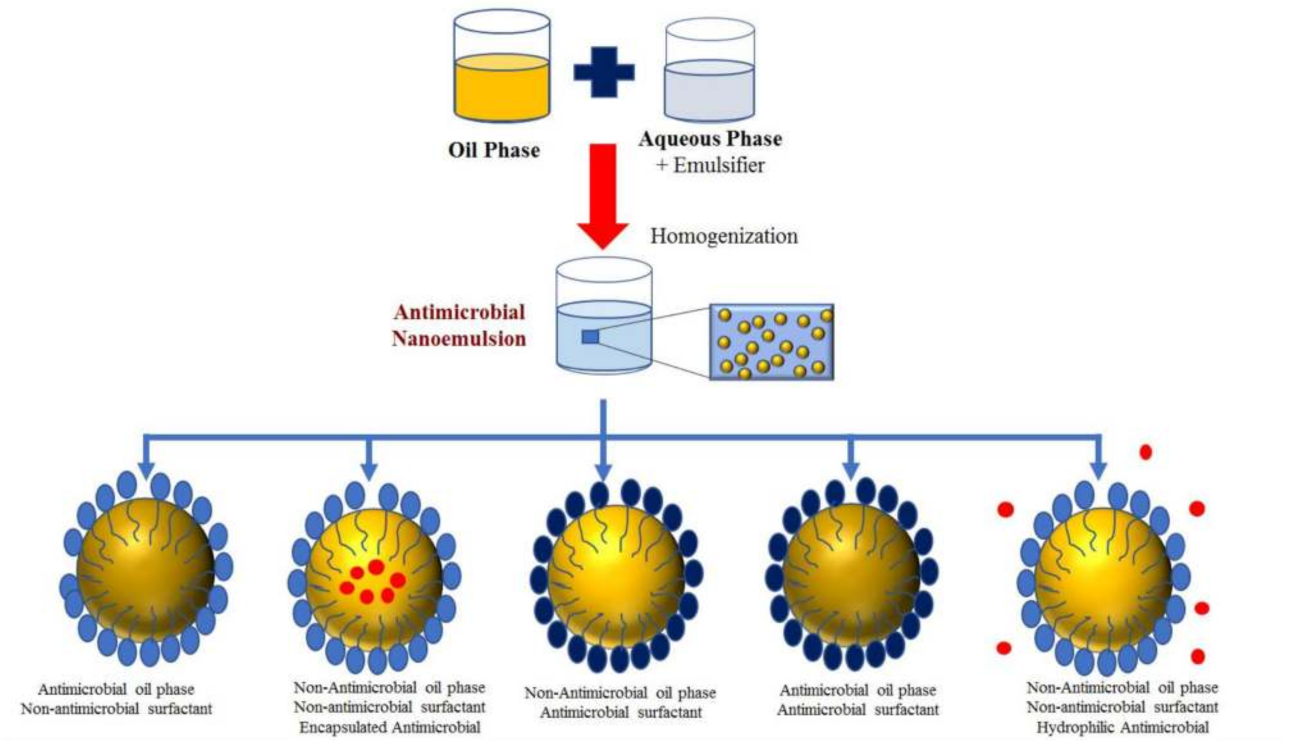

FIGURE 3 | Classification of antimicrobial nanoemulsions based on the location of the antimicrobial substances.

be designed to be metastable for sufficiently long periods for many commercial applications. In this section, we consider the ingredients and processing methods typically used to prepare oil-in-water nanoemulsions suitable for delivering natural plant-based antimicrobials.

Ingredients

Emulsifiers

Emulsifiers are required to facilitate the formation and to enhance the kinetic stability of nanoemulsions. Molecular emulsifiers have both hydrophilic and hydrophobic groups making them amphiphilic (Chakraborty and Dhar, 2017). A wide range of plant-based emulsifiers are available to form nanoemulsions, including proteins (like soy or pea protein), polysaccharides (like gum arabic or modified starch), phospholipids (like soy or sunflower lecithin), and surfactants (like quillaja saponin). In addition, synthetic surfactants are often used alone or in combination with these ingredients, such as Tween and Spans. In this section, some examples of common plant-based emulsifiers for food applications are given.

Legume proteins are abundant, inexpensive, sustainable, and have low allergenicity, thus possessing many of the desirable functional traits required for food applications (Aberkane et al., 2014). Many pulses proteins exhibit good surface-active molecules and can act as effective emulsifiers to form and stabilize nanoemulsions (Can Karaca et al., 2015). For instance, vicilin and legumin are common globulins found in legume proteins (Donsì et al., 2010). Vicilin has been reported to be more surface active than legumin, which was attributed to its more flexible tertiary structure (Swanson, 1990). Pea, lentil, and faba bean proteins have been used as effective emulsifiers for the formulation of 10 wt% algae nanoemulsions using microfluidization (Gumus et al., 2017). Among these three proteins, the lentil protein showed the best efficacy in terms of the providing resistance to environmental stresses such as pH, ionic strength, and temperature changes.

Soy protein, a rich source of essential amino acids, is collected from dehulled and deoiled soybean meal. It is a mixture of surface active globular proteins that can be used to form nanoemulsions to encapsulate bioactive agents (Chakraborty and Dhar, 2017). Soy proteins are abundant, inexpensive, biodegradable, and can exhibit good functional attributes, provided they are not denatured or aggregated prior to use. Soybean protein has been successfully used to fabricate perilla oil nanoemulsions using high-pressure homogenization (Hu et al., 2020). Similarly, sesame protein has been shown to be an effective emulsifier for forming fish oil nanoemulsions (Dey et al., 2018).

Stabilizers

The formulation of stable and efficacious antimicrobial nanoemulsions often requires the utilization of various other stabilizers, including ripening inhibitors, texture modifiers, weighting agents, interfacial stabilizers, and antioxidants. A number of the most important types of stabilizers are briefly reviewed here.

Ripening Inhibitors

Some antimicrobial nanoemulsions, especially those containing pure essential oils, are highly unstable due to a phenomenon known as Oswald ripening. This process occurs due to the net movement of oil molecules from smaller to larger oil droplets driven by differences in their curvatures. As a result, there is a net increase in the mean droplet size over time, which can accelerate creaming or coalescence. Ostwald ripening occurs particularly rapidly for polar oils (like essential oils) that have a relatively high water-solubility. This instability mechanism can be inhibited by adding hydrophobic substances that have a very low water-solubility into the oil phase of a nanoemulsion (Wooster et al., 2008). The origin of this inhibitory effect is due to entropy of mixing (McClements, 2005, 2011; Wooster et al., 2008; McClements and Rao, 2011). Typically, ripening inhibitors are highly hydrophobic substances like corn oil (Chang et al., 2012), sunflower oil (Donsì et al., 2012), medium-chain triglyceride oil (Liang et al., 2012). As an example, Ostwald ripening can be prevented in thyme oil nanoemulsions by adding a sufficiently high concentration of corn oil as a ripening inhibitor, but if too much corn oil is added it reduces the potency of the essential oil (Chang et al., 2012). Consequently, oil phase composition of nanoemulsions must be carefully formulated.

Texture Modifiers: Thickening and Gelling Agents

The stability of antimicrobial nanoemulsions can also be improved by adding texture modifiers to thicken or gel the aqueous phase, thereby inhibiting droplet movement. The most commonly used texture modifiers are polysaccharides and proteins. Thickening agents greatly increase the viscosity of aqueous solutions due to their large molecular dimensions, whereas gelling agents provide semi-solid characteristics to aqueous solutions by forming a 3D network of cross-linked molecules.

Carboxymethyl cellulose (CMC), a chemical derivative of cellulose is an anionic thickening agent that is widely used as a stabilizer in emulsions and nanoemulsions (Nussinovitch, 1997; Bayarri et al., 2009). It is colorless, odorless, palatable, and physiologically inert, which makes it suitable for use in fruit juices and soft drinks to inhibit gravitational separation (Mirhosseini et al., 2008; Bayarri et al., 2009). The impact of CMC on the flow behavior of food emulsions has been characterized using rheological methods (Arancibia et al., 2013). Sodium alginate has been used as a thickening agent and stabilizer in antimicrobial lemongrass oil nanoemulsions (Salvia-Trujillo et al., 2013). Pectin has also been added to oregano, thyme, mandarin, and lemongrass oil nanoemulsions as a thickening or gelling agent to improve their stability (Guerra-Rosas et al., 2016).

Interfacial Stabilizers

The stability of antimicrobial nanoemulsions can be improved using interfacial engineering approaches. In particular, electrostatic deposition methods have been used to coat oil droplets with polysaccharides, which increase the steric and electrostatic repulsion between them, thereby inhibiting aggregation. As an example, the storage stability of thymol nanoemulsions was increased by coating whey protein-coated oil droplets by chitosan hydrochloride (Li et al., 2021).

Weighting Agents

Weighting agents are added to the oil phase of nanoemulsions to inhibit gravitational separation, i.e., creaming and sedimentation (McClements, 2016). These substances are usually hydrophobic substances that have an appreciably higher density than water and can be dissolved in the oil phase prior to homogenization, such as brominated vegetable oil, ester gum, damar gum, and sucrose acetate isobutyrate. These weighting agents have all been shown to increase the creaming stability of nanoemulsions by matching the density of the oil phase to that of the water phase, thereby reducing the gravitational driving force for separation (Chanamai and McClements, 2000).

Antioxidants

Essential oils may oxidize during storage, thereby losing their antimicrobial activity. Consequently, it is often important to add natural antioxidants to inhibit their oxidation. For instance, quercetin has been reported to exhibit good antioxidant activity in nanoemulsions (Retta et al., 2012). Similarly, curcumin has been shown to reduce oxidation in nanoemulsions (Sanidad et al., 2019).

Nanoemulsion Formation

Oil-in-water nanoemulsions are thermodynamically unstable/kinetically stable colloidal dispersions containing oil droplets with relatively small diameters, i.e., $d < 200$ nm (Salvia-Trujillo et al., 2016). They can be produced using two different approaches: (i) low-energy methods and (ii) high-energy methods (Jiang et al., 2020). Low-energy methods produce small oil droplets using the internal chemical energy of the system, With or without gentle stirring (Ren et al., 2019). In contrast, high-energy methods produce small oil droplets by applying intense disruptive forces that break up the oil and water phases e.g., microfluidizers, high-pressure homogenizers, and sonicators (Ren et al., 2019; Jiang et al., 2020). The low-energy methods require no specialized equipment, but typically require high concentrations of synthetic surfactants (Borrin et al., 2016).

Low-Energy Emulsification Methods

Low-energy methods of preparing nanoemulsions include spontaneous emulsification (SE), phase inversion composition (PIC), phase inversion temperature (PIT), and emulsion inversion point (EIP) methods (Salvia-Trujillo et al., 2016). These methods are all based on the spontaneous formation of small oil droplets when the system composition or environmental conditions are altered in a specific way (Anton et al., 2008). The size and stability of the oil droplets produced depends on the type and concentration of surfactants and oils used, which should therefore be carefully optimized (Bouchemal et al., 2004). As an example, antimicrobial black pepper oil nanoemulsions have been prepared using the PIT method with Tween 80 as a non-ionic surfactant (Vinh et al., 2020). In this case, a mixture of water (86%), surfactant (9.7%), and oil (4.3%) was heated to 75°C for 30 min and then rapidly cooled to 5°C for 15 min, which led to the spontaneous formation of nanoemulsions. Moraes-Lovison et al. (2017) produced oregano oil nanoemulsions using the PIT method, which exhibited good antimicrobial activity against *Staphylococcus* and *E. coli.* The incorporation of these nanoemulsions into chicken pâté did not change the desirable physicochemical characteristics of the product. The EIP method has been used to form nanoemulsions containing a mixture of clove and cinnamon oil, which were found to act as a good food preservative due to their antimicrobial activity (Zhang et al., 2017). In another study, Chang et al. (2013) formulated antimicrobial carvacrol oil nanoemulsions using the SE method. The same method was also used to create antimicrobial nanoemulsions from a mixture of cinnamon and coconut oils (Yildirim et al., 2017). Finally, Marei et al. (2018) showed that chitosan/citral oil nanoemulsions formulated using a low-energy method exhibited potent antimicrobial activity against *Erwinia carotovora*, *Aspergillus niger,* and *Rhizopus stolonifer*.

High-Energy Emulsification Methods

Industrially, nanoemulsions tend to be produced by high energy methods such as high-pressure homogenization, microfluidization, and sonication (Salvia-Trujillo et al., 2016). In sonication, an ultrasonic probe placed in a mixture of oil, water, and emulsifier is used to generate intense disruptive forces (Jafari et al., 2008). In particular, microbubbles are created within the mixture due to cavitation effects due to by the rapid pressure fluctuations caused by the high-intensity ultrasonic waves, which mix the oil and water phases together and convert large droplets into smaller ones (Modarres-Gheisari et al., 2019). High-pressure homogenization uses a pump to force a coarse emulsion through a small valve, which generates intense shear, cavitation, and turbulent forces, thereby breaking down the large droplets (Modarres-Gheisari et al., 2019). Ma et al. (2017) have used this approach to prepare curcumin loaded-nanoemulsions from various kinds of oils and emulsifiers. Microfluidizers use a pump to force a coarse emulsion through two separate channels, which are then made to impinge on each other so that the fluids collide with each other at high velocity, thereby leading to the disruption of the large oil droplets (Bai and McClements, 2016).

High-intensity methods have been used to create various kinds of antimicrobial nanoemulsions. Sonication has been used to create antimicrobial nanoemulsions from *Cleome viscosa* essential oil and Tween 80, which were shown to be effective against *Streptococcus pyogenes, S. aureus*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa* (Krishnamoorthy et al., 2018). Sonication has also been used to produce chitosan/eugenol oil nanoemulsions that exhibited strong antimicrobial activity (Shao et al., 2018). The same method has also been used to prepare citral oil nanoemulsions that were shown to exhibit potent antimicrobial effects against several bacterial strains (Lu et al., 2018). Sonication has also been used to form antimicrobial nanoemulsions from lemon myrtle and anise myrtle essential oils (Nirmal et al., 2018), geraniol and carvacrol (Syed and Sarkar, 2018), and thyme oil (Moghimi et al., 2016).

High-pressure homogenization has been used to prepare black pepper oil nanoemulsions that exhibited antimicrobial efficacy against bacterial pathogens in aquaculture (Swathy et al., 2018). Similarly, black pepper and cinnamon oil nanoemulsions produced by the same method have been shown to exhibit an antimicrobial effect against disease-related pathogens and food spoilage bacteria (Jiménez et al., 2018). Microfluidization has been used to produce antimicrobial carvacrol nanoemulsions using a natural surfactant (quillaja saponin), leading to all natural plant-based formulations (Ryu et al., 2018). A simple high-shear mixer has been used to form eugenol and thymol oil nanoemulsions stabilized by a mixture of food-grade surfactants (lauric arginate and lecithin), which exhibited good antibacterial effects against *L. monocytogenes* and *E. coli* (Ma et al., 2016).

Microencapsulation

Spray-drying can be used to convert a nanoemulsion into a fine powder, which improves its handling, storage, transport, and stability (Gharsallaoui et al., 2007). A nanoemulsion is first formed in a fluid state and then passed through a nozzle that sprays it into a hot chamber, which leads to rapid evaporation of the water phase. Nielsen et al. (2016) showed that spray drying could also be used to convert isoeugenol oil nanoemulsions into powders, which had high essential oil loading capacities and exhibited good antimicrobial activity against both Gram-positive and Gram-negative bacteria. The same authors showed that these nanoemulsions could disrupt the biofilms formed by pathogenic bacteria (Nielsen et al., 2017). Spray drying has also been used to convert eugenol nanoemulsions into powders (Talón et al., 2019). The powders produced had high encapsulation efficiencies (95–98%) and exhibited strong antibacterial activity against *E. coli* when dispersed in water.

Droplet Properties

The physicochemical properties, stability, and efficacy of antimicrobial nanoemulsions can be altered by varying oil droplet properties (Buranasuksombat et al., 2011), such as their composition, concentration, interfacial properties, and physical state. For instance, reducing the droplet size reduces the opacity and increases the resistance to gravitational separation and aggregation of nanoemulsions. In addition, the droplet characteristics impact how the oil droplets interact with the surfaces of microbes. For instance, the droplets can be made to stick to the surfaces of the microbes using electrostatic effects: positively charged oil droplets will be attracted to negatively charged microbe surfaces, thereby bringing them into close proximity. Consequently, it is important to carefully tailor the properties of the oil droplets for particular applications.

Physicochemical Properties and Stability

Nanoemulsions are thermodynamically unstable systems that breakdown by different physicochemical mechanisms. The main destabilization mechanisms of nanoemulsions are gravitational separation (creaming/sedimentation), flocculation, coalescence, and Ostwald ripening, which have been reviewed in detail elsewhere (McClements, 2005).

Gravitational Separation

A density difference normally exists between the oil and water phases in nanoemulsions, which promotes phase separation through gravitational forces. The density of oil droplets tends to be lower than that of water, thereby causing them to move upward due to creaming (Pathak, 2017). In the rare cases where the oil droplet density is higher than that of water, the droplets move downwards due to sedimentation. The rate of gravitational separation is governed by Stokes' Law, which states that the rate is proportional to the density different between the phases, the square of the droplet size, and the inverse of the continuous phase viscosity. Thus, nanoemulsion stability can be increased by reducing the particle size and density contrast, and increasing the aqueous phase viscosity.

Aggregation

Aggregation occurs when the attractive forces (usually van der Waals, hydrophobic, depletion, and bridging attraction) acting between the droplets outweigh the repulsive forces (usually steric and electrostatic repulsion) (McClements, 2005). It is therefore important to identify the dominant colloidal interactions present within nanoemulsions and then reduce the attractive forces and/or increase the repulsive forces by changing the composition or structure of the system. Droplet aggregation may occur due to coalescence or flocculation depending on the nature of the forces involved. Coalescence involves the merging together of smaller droplets into larger ones. Flocculation involves two or more droplets coming together and forming clusters. Often, flocculation is a precursor for coalescence since it brings the droplets close together for prolonged periods. In dilute nanoemulsions, droplet aggregation usually accelerates creaming or sedimentation due to the increase in particle size. Eventually, coalescence may lead to the formation of a separate layer of oil on top of the nanoemulsion ("oiling off"). In concentrated nanoemulsions, droplet flocculation can actually inhibit gravitational separation and lead to the formation of a semi-solid texture because of the formation of a 3D particle network that extends throughout the sample.

Ostwald Ripening

This mechanism leads to a net increase in droplet size due to diffusion of oil molecules from small to large droplets driven by differences in droplet curvature (McClements, 2005). The rate of Ostwald ripening increases as the water-solubility of the oil phase increases, and is therefore more important for polar oils, such as the essential oils often used as plant-based antimicrobial agents. Consequently, it is important to prevent this mechanism by adding ripening inhibitors (see section Ripening Inhibitors). These ripening inhibitors are strongly hydrophobic molecules that have a very low solubility in water. As a result, they cannot move from one droplet to another very quickly, if at all. Thus, as Ostwald ripening proceeds there is a difference in composition between the small and large oil droplets, which generates an osmotic stress that opposes further exchange of oil molecules between the droplets due to an entropy of mixing effect. Eventually, droplet growth due to Ostwald ripening is therefore inhibited.

DELIVERY OF PLANT ANTIMICROBIALS USING NANOEMULSIONS

Application in Agriculture

Pesticides are used to improve the quality and yield of crops by reducing their susceptibility to microorganisms, insects, herbivores, and other invasive species (weeds) (Zhang et al., 2015; McClements, 2020). These pesticides may be fungicides, nematicides, insecticides, and herbicides depending on their target (Feng et al., 2018). The use of synthetic chemical pesticides in excessive quantity can damage the environment and pose a risk to human health. Consequently, there is interest in developing natural plant-based pesticides that are less toxic. The hydrophobic nature of many natural pesticides, however, means they have a low water-solubility, and so it is difficult to get the required quantity to the site of action. They can sometimes be converted into solids, but powdered or granular forms of these pesticides often have limitations due to their large particle size, poor adhesion properties, and low solubility characteristics (Mulqueen, 2003).

For these reasons, there is growing interest in the use of colloidal forms of plant-based pesticides, such as antimicrobial nanoemulsions (McClements, 2020). These nanoemulsions should be carefully designed to protect the antimicrobials from evaporation or chemical degradation during storage and application, while still remaining efficacious against the target spoilage or pathogenic microorganisms. The use of nanoemulsions can improve the potency and/or reduce the required dose of plant-based antimicrobials, which may reduce their potential toxicity (Ibrahim, 2020). For example, insecticidal nanoemulsions have been formulated from a hexane-soluble fraction of *Manilkara subsericea* for use against an important cotton pest (*Dysdercus peruvianus*) (Fernandes et al., 2014). This study showed that the nanoemulsions were effective against the pests, while also having a lower impact on the environment and on non-targeted organisms. Nanoemulsions containing neem oil were shown to be effective against two insects, the rice weevil (*Sitophilus oryzae*) and the red flour beetle (*Tribolium castaneum*) that infect important commercial crops (Choupanian et al., 2017). The droplet size of the nanoemulsions influenced the mortality rate, with smaller particle sizes being more effective. Antimicrobial nanoemulsions formulated from *Mentha piperita* oil have been shown to be effective insecticides against cotton aphids (Heydari et al., 2020). Nanoencapsulated *Melissa officinalis* L. oil was reported to exhibit strong insecticidal activity against *Tribolium castaneum* Herbst and could be used as an ecofriendly and safe plant-based pesticide (Upadhyay et al., 2019). These studies suggest that plant-based nanopesticides could be a viable alternative to conventional chemical pesticides.

Application in Foods

There has been a growing demand for healthier and safer food products by consumers, which has led manufacturers to reformulate their products with more natural ingredients (Das et al., 2019). However, these ingredients must still exhibit the required functional attributes. Foods with high moisture contents, nutrient levels, and pH values tend to spoil easily and cannot normally be stored for long periods. Post-harvest handling and processing operations often increase the chances of microbial contamination and growth, thereby reducing food quality and shelf-life (Hygreeva and Pandey, 2016; Das et al., 2019). Food-borne pathogens such as *Clostridium botulinum, Salmonella, S. aureus, Campylobacter jejuni, B. cereus, L. monocytogenes, Cryptosporidium,* and *E. coli* O157:H7 may cause foodborne illnesses (Arshad and Batool, 2017). Natural antimicrobials are therefore being developed to reduce microbial contamination and prolong the shelf-life of these products (Barros-Velazquez, 2016). A number of natural antimicrobial agents have been shown to be promising alternatives to synthetic ones (Donsì and Ferrari, 2016). Again, many of these antimicrobial agents are hydrophobic substances that only have limited water-solubility and have poor chemical stability. These problems can often be overcome by using well-designed nanoemulsions as delivery systems.

Antimicrobial nanoemulsions have been reported to be able to suppress microbial growth, maintain quality, and prolong the shelf-life of many foods (Salvia-Trujillo et al., 2015; Das et al., 2020a). These systems have been shown to be effective against both Gram-positive and Gram-negative bacteria (Sutcliffe et al., 2008; Ferreira et al., 2010), which has been attributed to their ability to damage the structure and function of microbial cell walls (Jones et al., 1997; Baker et al., 2003) or to prevent biofilm formation by interrupting intercellular signaling (Joe et al., 2015). The strong antimicrobial activity of nano-emulsified version of plant-based antimicrobials like essential oils and other phytochemicals, such as carvacrol, cinnamaldehyde, thymol, limonene, eugenol, and curcumin, have been demonstrated against a broad spectrum of spoilage pathogens in various food systems (Donsì et al., 2012; Salvia-Trujillo et al., 2016; Quinto et al., 2019). Representative examples of studies on plant-based antimicrobial nanoemulsions are summarized in **Table 3**.

Meat and Meat Products

Some of the major species of microorganisms contaminating meat and meat products are *Salmonella, S. aureus, C. jejuni, E. coli* O157:H7, and *L. monocytogenes,* with the latter being the most commonly detected (Kaur and Kaur, 2020). Numerous studies have shown that plant-based antimicrobial nanoemulsions have promise to control microbial growth in these products. These nanoemulsions may be applied to meat and fish products as additives, dips, sprays, coatings, or films (Wu et al., 2016b; Pabast et al., 2018).

Oregano oil nanoemulsions have been incorporated into chicken pate to significantly inhibit the growth of *S. aureus* and *E. coli,* thereby extending the shelf-life (Moraes-Lovison et al., 2017). Balta et al. (2017) reported that geraniol- and linalool-loaded nanoemulsions inhibited the growth of *E. coli* K12, *Listeria innocua,* and *Pseudomonas lundensis* in a simulated meat system. Ghaderi-Ghahfarokhi et al. (2017) reported a significant decrease in *S. aureus*, *Enterobacteriaceae,* yeast, and mold when beef patties were treated with chitosan/cinnamon oil nanoemulsions. Curcumin-loaded nanoemulsions containing pectin-coated oil droplets were shown to inhibit the growth of psychrophilic bacteria, yeast, and mold in refrigerated chicken fillets (Abdou et al., 2018). Microbial growth has also been inhibited in refrigerated Turkey meat treated with essential oil nanoemulsions containing *Zataria* Boiss (*ZEO*) and *Bunium persicum* Boiss (*BEO*), as well as chitosan (Keykhosravy et al., 2020). These authors reported that 1% *ZEO* nanoemulsions exhibited strong inhibitory effects on total viable bacteria, total psychrophilic bacteria, *Pseudomonas* spp., *Enterobacteriaceae,* lactic acid bacteria, *S. Enteritidis*, and *L. monocytogenes*. As a result, the shelf-life increased from 6 days in the untreated meat to 15–18 days in the nanoemulsion-treated meat. As well as improving the safety and shelf-life of meat products, plant-based antimicrobial nanoemulsions have also been shown to improve their physicochemical and sensory properties (Das et al., 2020a).

Plant-based antimicrobial nanoemulsions must be carefully formulated for application in meat products. Numerous studies have reported that the efficacy of plant-based antimicrobials may be reduced in meat due to their interaction with meat components, such as fat droplets and proteins (Das et al., 2020a; Kaur and Kaur, 2020).

Milk and Milk Products

The dairy industry also faces challenges with spoilage and pathogenic organisms (Boor et al., 2017). As a result, they may also benefit from the utilization of antimicrobial nanoemulsions (Kaur and Kaur, 2020). Thymol oil nanoemulsions stabilized by casein are effective antimicrobial agents in milk products (Pan et al., 2014). Thymol nanoemulsions stabilized by a

TABLE 3 | Delivery of various kinds of plant-based antimicrobials using nanoemulsions in different food systems.

Plant antimicrobial	Nanoemulsion type	Method of nanoemulsion preparation	Food system	Key findings	References
Oregano oil	Nanoemulsions containing mandarin fiber	Microfluidization	Low-fat cheese	• Inhibited *Staphylococcus aureus* population from 6.0 to 4.6 log CFU/g after 15 days. • Decreased psychrophilic bacteria, molds and yeasts growth during storage	Artiga-Artigas et al., 2017
Satureja khuzestanica oils	Chitosan coatings loaded with free or nano-encapsulated oil	Thin-film hydration-sonication method	Lamb meat	• Effectively retarded microbial growth and chemical spoilage • Extended the shelf-life of lamb meat by maintaining prolonged antimicrobial and antioxidant activity • Improved sensory attributes	Pabast et al., 2018
Zataria multiflora Boiss and *Bunium persicum* Boiss oils	Nanoemulsions loaded into chitosan-based coatings	Sonication	Turkey meat	• Reduced total viable bacteria, total psychrophilic, *Pseudomonas* spp., Enterobacteriaceae, lactic acid bacteria, and yeast and mold count than control samples • Increased the shelf-life significantly of treated samples up to 15–18 days	Keykhosravy et al., 2020
Oregano oil	Nanoemulsions (5–7 g oil/100 g nanoemulsion)	Phase inversion temperature method	Chicken pate	• Exhibited good antibacterial action against *S. aureus* and *Escherichia coli* with minimum inhibitory concentration 0.56 and 0.60 mg/ml, respectively • Improved shelf-life by controlling the microbial growth	Moraes-Lovison et al., 2017
Sunflower oil	Nanoemulsions based on sunflower oil	Sonication	Farmed sea bass and gilthead sea bream fillets	• Inhibited the bacterial growth • Prolonged the shelf-life as well as improved the quality of fillets during storage	Yazgan et al., 2017
Thymol nanoemulsions	Nanoemulsions stabilized by gelatin and lecithin	Homogenization and Sonication	Milk and cantaloupe juice	• Inhibited the *Listeria monocytogenes* by 5 and 3 log CFU/mL in 2% reduced fat and full fat milk after 48 h, respectively • Both thymol emulsions more effective than free thymol against *L. monocytogenes* and *E. coli* O157:H7 in milk • Could be used as novel antimicrobial preservatives to enhance the food safety	Xue et al., 2017
Thyme oil	Nanoemulsions	Sonication	Fish	• Effectively inhibited the food-borne pathogens by damaging bacterial cell membranes • Nanoemulsion formulation increased the antibacterial activity of thyme • Have potential as an alternative antimicrobial agent in processed or packaged fish or food products.	Ozogul et al., 2020
Geraniol and linalool	Nanoemulsions prepared with geraniol or linalool	Sonication	Meat	• Significantly reduced *E. coli K12,* and *Listeria innocua* counts • Could be used as promising antimicrobial system for food preservation and food safety	Balta et al., 2017
Cinnamon, garlic or sunflower oil	Nanoemulsions loaded into pectin-based coatings	Emulsion inversion point method	Chicken fillets	• Coating with nanomeulsion reduced the total plate count, psychrophilic bacteria, yeast, and mold growth • Effectively checked the deterioration of fillets texture • Prolonged shelf-life up to 12 days	Abdou et al., 2018

(Continued)

TABLE 3 | Continued

Plant antimicrobial	Nanoemulsion type	Method of nanoemulsion preparation	Food system	Key findings	References
Cinnamon oil	Nanoemulsion-loaded pullulan coatings	High-pressure homogenization and Sonication	Fresh strawberries	• Exhibited strong antimicrobial behavior against molds and bacteria during room storage • Decreased loss of firmness, fruit mass, titratable acidity, and total soluble solids of strawberries • Enhanced shelf-life	Chu et al., 2020
Citrus oil	Nanoemulsions containing chitosan	Emulsion-ionic gelation technique	Fresh silvery pomfret fillets	• Successfully checked the microbial growth • Reduced the biochemical changes • Extended shelf-life from 12 to 16 days • Have potential as an effective preservative for marine fish	Wu et al., 2016a
Eugenol	Nanoemulsions stabilized by Tween 80	Sonication	Fresh orange juice	• Exhibited bactericidal activity against *S. aureus* • Reduced native bacterial population up to 24 h (4°C storage) and 6 h (25°C storage) • Exhibited better antibacterial activity than sodium benzoate	Ghosh et al., 2014
Thymol	Thymus nanoemulsion (*NE-Thy*) loaded into quinoa protein-chitosan films	Spontaneous emulsification (SE) method and/or Sonication	Cherry tomatoes	• NE-*Thy* prepared with SE exhibited antifungal activity against *Botrytis cinerea* • The edible coating of NE-SE incorporated quinoa protein-chitosan film inhibited *B. cinerea* growth on cherry tomatoes • Can be used as edible antifungal bioactive film for improving fruit shelf-life	Robledo et al., 2018
Oregano oil	Oregano oil nanoemulsions stabilized by Tween 80	Sonication	Fresh lettuce leaves	• Inhibited the growth of *Salmonella Typhimurium, E. coli O157:H7,* and *L. monocytogenes* on lettuce • Effective natural alternative to different chemicals for food safety control	Bhargava et al., 2015
Oregano and clove bud oil	Nanoemulsions loaded into methylcellulose-based film	Sonication	Bread	• Sonication improved plasticizing behavior of film and antimicrobial activity of essential oil • Inhibited common spoilage fungi • Prolonged bread shelf-life	Otoni et al., 2014
Rosemary, cinnamon, and oregano oils	Nanoemulsions stabilized by Tween 80	Sonication	Fresh celery	• Reduction in bacterial count in celery • Inhibited *E. coli* and *L. monocytogenes* growth	Dávila-Rodríguez et al., 2019
Ginger oil	Nanoemulsions loaded into caseinate coatings	Sonication	Fresh chicken breast fillet	• Inhibited growth of *L. monocytogenes* and *S. Typhimurium* • Reduction in total aerobic psychrophilic bacterial, yeasts and molds in chicken fillet during refrigeration • Increased shelf-life of chicken breast fillets	Noori et al., 2018
Star anise oil	Nanoemulsions loaded into soy protein-based coating	Sonication	Yao meat products	• Coatings improved total volatile basic nitrogen and pH significantly • Coatings inhibited bacterial growth during storage at 4°C • Coatings improved odor, color and sensory acceptability • Extended shelf-life of Yao meat products up to 16 days	Liu Q. et al., 2020

(Continued)

TABLE 3 | Continued

Plant antimicrobial	Nanoemulsion type	Method of nanoemulsion preparation	Food system	Key findings	References
Lemongrass oil	Nanoemulsions loaded into alginate-based films	Microfluidization	Fresh-cut Fuji apples	• Strong inhibition of *E. coli* and diminished growth of psychrophilic bacteria during storage • Inhibited the habitat microbes of fresh-cut Fuji apples during 2 weeks of storage	Salvia-Trujillo et al., 2015
Mandarin oil	Nanoemulsions loaded into chitosan-based coating	High pressure homogenization	Fresh green beans	• Synergistic antimicrobial effect of bioactive coating and γ-irradiation observed during storage • Bioactive coating along with UV-C treatment reduced *L. innocua* population without affecting the firmness and color of green beans during storage	Severino et al., 2014
Carvacrol	Nanoemulsions stabilized by Tween 80	High pressure homogenization, sonication	Shredded cabbages	• Carvacrol nanoemulsion inhibited growth of *E. coli* and *Pichia pastoris* • Carvacrol nanoemulsion had strongest effect in reducing aerobic psychrotrophic and mesophilic bacteria on shredded cabbage • Antimicrobial effect of the treatment solution lasted for 2 days	Sow et al., 2017
Zataria multiflora Boiss oil (ZEO)	Nanoemulsions loaded into alginate-based films	Sonication	Fresh trout	• Reduced (1% w/v ZEO) total viable, hydrogen sulfide-producing bacteria, total psychrophilic, and *Enterobacteriaceae* count • All the treatment types significantly reduced the microbial population in fresh trout fillets during storage for 16 days at 4°C	Khanzadi et al., 2020
Curcumin, gallic acid, quercetin	Nanoemulsions loaded into gelatin-based films	High pressure homogenization	Fresh broiler meat	• Curcumin loaded gelatin films inhibited *S. Typhimurium* and *E. coli* growth • Prolonged shelf-life of meat for about 17 days in contrast to 10 days for control	Khan et al., 2020
Mustard oil	Nanoemulsions stabilized by Tween 80	Sonication	Turkey meat	• Reduced molds, yeast, total psychrotrophic and mesophilic bacteria during storage • Shelf life of Turkey meat was increased to 10–15 days • Improved sensory attributes, especially odor and overall shelf-life of meat during storage at 4°C	Milani et al., 2020
Ferulago angulata oil	Nanoemulsion-loaded chitosan-based coating	Sonication	Rainbow trout fillets	• Improved antimicrobial activity against *Shewanella putrefaciens* and *Pseudomonas fluorescens* • Offered better color, texture and overall acceptability in treated samples • Coating @ 3% successfully retarded the bacterial growth and improved shelf-life of fillets in rainbow trout fillets	Shokri et al., 2020
Ziziphora clinopodioides essential oil (ZCEO)	Nanoemulsion loaded chitosan-based coating	Sonication	Fresh beef	• Nanoemulsion of chitosan-ZCEO-nisin significantly inhibited the growth of *E. coli* O157:H7 in beef samples than control • Effective in controlling pathogenic bacteria in beef during storage	Khanzadi et al., 2019

mixture of gelatin and lecithin have been shown to exhibit strong antibacterial activity against *L. monocytogenes* and *E. coli* O157:H7 in milk and cantaloupe juice (Xue et al., 2017). In some studies, the fat level in dairy products has been shown to influence the antibacterial activity of nanoemulsions. For instance, a higher inhibition of *L. monocytogenes* was reported in reduced fat milk (5 log CFU/ml) than in full fat milk (3 log CFU/ml) after 48 h storage (Xue et al., 2017). This effect can be attributed to partitioning of more of the hydrophobic antimicrobial agents into the milk fat globules in the full fat milk.

Nanoemulsion-based antimicrobial edible coatings prepared from oregano oil were shown to extend the shelf-life of low-fat cheese by inhibiting the growth of bacteria, molds and yeasts (Artiga-Artigas et al., 2017). In another study, encapsulation of basil (*O. basilicum*), mint (*M. piperita*), sea buckthorn (*Hippophae rhamnoides*), and fennel (*Foeniculum vulgare*) oils in sodium alginate hydrogels improved the quality characteristics and shelf-life of yogurt, which was attributed to their antimicrobial and antioxidant effects (Constantinescu et al., 2019).

Fish and Fish Products

In a recent study, thyme oil nanoemulsions were shown to exhibit antimicrobial activity against various food-borne pathogens (*S. Paratyphi A, S. aureus*, *K. pneumoniae,* and *E. faecalis*) and spoilage bacteria (*Pseudomonas luteola, Photobacterium damselae*, *Vibrio vulnificus*, *E. faecalis*, *Serratia liquefaciens*, and *Proteus mirabilis*) associated with fish and fish products (Ozogul et al., 2020). The antimicrobial activity of these nanoemulsions was attributed to their ability to damage the bacterial cell membranes. Similarly, sage oil nanoemulsions were shown to exhibit strong antimicrobial activity against food-borne pathogens and spoilage organisms, such as *E. faecalis*, *K. pneumoniae*, *S. Paratyphi A,* and *S. aureus*, with MIC values in the range 6.25–12.5 mg/ml (Yazgan, 2020). In another study, Joe et al. (2012) reported a significant reduction in heterotrophic and lactic acid bacteria populations, and an extension in shelf-life, for fish steaks treated with sunflower oil nanoemulsions. Similarly, sea bream and sea bass fillets treated with sunflower oil nanoemulsions had a lower microbial load and longer shelf-life than control samples during refrigerated storage (Yazgan et al., 2017). These studies show the potential of plant-based antimicrobial nanoemulsions as preservatives to improve the safety, quality, and shelf-life of fish products (Acevedo-Fani et al., 2017a).

Fruits and Vegetables

Fresh or only minimally processed fruits and vegetables are linked to foodborne diseases because microbial contaminants may be present. Numerous studies have shown that plant-based antimicrobial nanoemulsions can be used to improve the quality and safety of fruits and vegetables (Prakash et al., 2018). For instance, chitosan-based edible coatings containing mandarin oil nanoemulsions exhibited antimicrobial activity against *L. innocua* in green beans (Severino et al., 2014). Similarly, thymol nanoemulsions inhibited the growth of molds (*B. cinerea*) on refrigerated tomatoes (Robledo et al., 2018). In another study, edible coatings containing lemon oil nanoemulsions improved the safety and quality of freshly cut Fuji apples (Salvia-Trujillo et al., 2015), which was attributed to their ability to inhibit microbial growth. Oregano oil nanoemulsions have been reported to reduce the growth of *S. Typhimurium*, and *E. coli* O157:H7 on fresh lettuce by 3.4, 2.3, and 3.1 log CFU/g, respectively (Bhargava et al., 2015). Similarly, geraniol- and carvacrol-loaded nanoemulsions significantly inhibited the growth of Gram-positive bacteria (*B. cereus*) and Gram-negative bacteria (*E. coli*) (Syed and Sarkar, 2018). These results highlight the potential of plant-based nanoemulsions to improve the quality, safety, and shelf-life of minimally processed or fresh fruits and vegetables.

Cereals and Cereal-Based Products

Both raw and processed cereal-based products are prone to spoilage microorganisms, such as bacteria, yeast, and mold. This spoilage reduces the quality and safety of cereal products and causes food safety concerns. There have only been a few studies on the use of nanoemulsions to improve the efficacy of plant-based antimicrobial agents in cereal-based products (Kaur and Kaur, 2020). Methylcellulose films loaded with clove bud oil (*S. aromaticum*) or oregano oil (*Origanum vulgare*) nanoemulsions have been shown to inhibit the growth of yeasts and molds in sliced bread (Otoni et al., 2014). The antimicrobial activity of these systems increased as their droplet size decreased, which meant that a lower dose could be used for the nanoemulsions. *Myristica fragrans* oil encapsulated within chitosan nano-particles exhibited strong antimicrobial activity against *A. flavus* in stored rice (Das et al., 2020c). An α-terpineol-loaded chitosan nanoemulsion was reported to inhibit yeasts and molds growth, as well as aflatoxin B_1 production in stored maize (Chaudhari et al., 2020a). Similarly, eugenol and *Origanum majorana* L. oils loaded into nanoemulsions have been shown to exhibit antifungal and anti-aflatoxigenic activity, thereby improving the shelf-life of stored foods (Chaudhari et al., 2020b; Das et al., 2021a). In addition, methylcellulose-based films loaded with essential oil (oregano + thyme oil) nanoemulsions have been shown to exhibit antifungal activity in rice (Hossain et al., 2019). In particular, they exhibited strong activity against *A. niger, Aspergillus flavus, Aspergillus parasiticus, and Penicillium chrysogenum*.

SAFETY ASPECTS OF PLANT ANTIMICROBIAL BASED NANO-FORMULATIONS

As stated earlier, plant-based antimicrobials have been widely used as therapeutics, food preservatives, and biocides for many years. In the past two decades, many studies have focused on the efficacy, biological activity, and pharmacological potential of these antimicrobials (Juneja et al., 2012; Mickymaray, 2019). However, there is still a lack of detailed information about the potential toxicity of plant-based antimicrobial nanoemulsions (Chaudhry et al., 2017). Even so, some studies on the toxicological effects of specific formulations have been reported

(Carocho et al., 2015; Davidson et al., 2015; Jeevanandam et al., 2017). Some of the surfactants, excipients, and other additives used to formulate these nanoenabled antimicrobial systems may have toxic effects when used at sufficiently high concentrations (McClements, 2011; Simões et al., 2018). Furthermore, the small size of the droplets in nanoemulsions may alter their behavior within the human gut, which could alter their toxicity (McClements and Xiao, 2012; Ribeiro et al., 2017; Wani et al., 2018). For instance, they may be absorbed in different regions of the gastrointestinal tract, they may have different pharmacokinetic profiles, or they may interfere with the gut microbiome. There is clearly a need for a more comprehensive assessment of the potential toxicological effects of plant-based antimicrobial nanoemulsions, which would involve developing standardized methods for their proper physicochemical characterization, risk assessment, and exposure assessment (Hardy et al., 2018; Rasmussen et al., 2018). Government agencies like EFSA (European Food Safety Authorities) are developing principles for the safety evaluation and authorization of nano-based materials (Amenta et al., 2015), with the ultimate goal of facilitating the development of effective products that are also safe for use.

CONCLUDING REMARKS AND FUTURE OPPORTUNITIES

The use of synthetic compounds in food preservation and food safety applications has raised consumer concerns due to perceived adverse health effects, the development of antimicrobial resistance, and environmental pollution. To address these issues, the use of plant-based antimicrobial substances for food preservation has become an important area of research. Many of these substances cannot be used in their pure form because of solubility, stability, and activity issues. For this reason, nanoemulsion-based delivery systems are being designed to overcome these limitations, as well as to create targeted or controlled release systems. Nevertheless, these nanoemulsions must be carefully designed to ensure they have the required functional attributes, which involves tight control of their formulation and manufacture. In particular, the composition, concentration, size, and surface charge of the oil droplets must be designed for the specific application. One or more antimicrobial substances can be located within different locations within nanoemulsions: non-polar antimicrobials in the oil droplets; polar antimicrobials in the water phase; and, amphiphilic antimicrobials in the interfacial region.

Much of the research carried out so far has been on simple model systems. However, food matrix effects are known to have a major impact on the efficacy of antimicrobial nanoemulsions because of partitioning and interaction effects. Consequently, more studies should be carried out on the impact of plant-based antimicrobial nanoemulsions on the quality attributes, safety, and shelf-lives of real food products. In addition, more research is required to identify the mechanisms of action of different antimicrobials under different conditions, as well as their potential for causing toxicity.

AUTHOR CONTRIBUTIONS

DM planned and edited the final draft of manuscript. AD, PD, PN, and NC researched the literature and wrote first draft of manuscript. All authors contributed to the article and approved the submitted version.

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