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Nanoemulsions: Preparation, Structure, Functional Properties and their Antimicrobial Effects

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Abstract

Background and Objectives: Recently, due to the interest of healthy lifestyle demand for research on novel methods of increasing the shelf-life of food products without the necessity of using preservatives has extended rapidly in the world. Ability of nanoemulsions to improve global food quality has attracted great attention in food preservation. This is as a result of a number of attributes peculiar to nanoemulsions such as optical clarity, ease of preparation, thermodynamic stability and increased surface area. This review discusses the potential food applications of nanoemulsions as vehicles for the delivery of antimicrobial compounds. Moreover, the preparation, structure, and functional properties of nanoemulsions and their antimicrobial effects on foodborne pathogens and biofilms will be reviewed in detail. Antimicrobial nanoemulsions are formulated from the antimicrobial compounds that are approved by the Food and Drug Administration (FDA) for use in foods.

Results and Conclusion: The antimicrobial activity of nanoemulsions is nonspecific, unlike that of antibiotics, thus they have a broad-spectrum of antimicrobial activity against bacteria (e.g., *Escherichia coli*, Salmonella, and *Staphylococcus aureus*), enveloped viruses (e.g., HIV, and herpes simplex), fungi (e.g., Candida, Dermatophytes), and spores (e.g., anthrax) at concentrations that are nontoxic in animals (while limiting the capacity for the generation of resistance) and kill pathogens by interacting with their membranes. This physical kill-on-contact mechanism significantly reduces the possibility of the emergence of resistant strains. In general, more research is needed to improve the application processes of antimicrobial nanoemulsion, especially sensory aspects, to be appropriate for each product.

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1. Introduction

1.1. Objective

Nanoscalar particulate systems (e.g. nanoemulsion) have been shown to differ substantially in terms of their physicochemical properties from larger microscopic systems (e.g. emulsion) due to their submicron particle diameters [1]. Properties affected by the small particle diameters include particle–particle interactions, interaction with electromagnetic waves (e.g. light), interaction with biological tissue, crystallization processes, catalytic activities, and others [2]. For food manufacturers, introduction of these nano-scalar systems into foods can result in favorable changes in food quality attributes such as appearance, texture, flavor and aroma. In the case of biologically or biochemically active compounds such as antimicrobials, nutraceuticals and antioxidants, substantial alterations in the functionalities of the encapsulated compounds may be seen [3, 4]. Nanoemulsions are heterogeneous systems consisting of two immiscible liquids, with one liquid phase being dispersed as nanometric droplets into another continuous liquid phase and stabilized through an appropriate emulsifier. In particular, oil in water (O/W) nanoemulsions, which are of prevalent interest for food delivery systems, are composed of oil droplets dispersed in an

aqueous medium and stabilized by surfactants approved for human consumption and common food substances that are Generally Recognized as Safe (GRAS) by the FDA, with mean droplet size typically ranging from 20 to 200 nm [1]. The selection of emulsifier type is important to control the interfacial properties (charge, thickness, droplet size, and rheology), as well as the response of nanometric oil droplets to the environmental stresses such as pH, ionic strength, temperature, and enzyme activity [5].

Not only all the emulsions used to change the texture, appearance and flavor of foods, but also the capacity of nanoemulsions to dissolve large quantities of lipophilic functional compounds such as antioxidants, flavors and nutraceuticals along with their mutual compatibility and ability to protect the bioactive compounds from degradation reactions make them ideal vehicles for their functional performances [5]. Additionally, the lack of flocculation, sedimentation and creaming combined with a large surface area and free energy offers obvious advantages over emulsions of larger particle size for this route of administration [6]. Their very large interfacial area positively influences the bioactive compounds transport and their delivery, along with targeting them to specific sites. Moreover, nanoemulsions may not scatter light strongly in the visible region, and can thus be transparent [7].

1.2. Global scenario

To develop a mature knowledge base of nanoscale processing, product formulation and shelf stability of foods with enhanced nutrition value is a part of individualized health management practices. Research in the area of food safety has become particularly urgent due to a lack of new preventative measures to control foodborne illnesses [8, 9]. Two high-profile recalls occurred in 2006; a recall of 100% carrot juice prompted by three cases of botulism following consumption of temperature-abused juice [10, 11], and the removal of all fresh bagged spinach from the store shelves after 199 people across 26 states became infected with *Escherichia coli* O157:H7 [12,13]. Laboratory-confirmed cases of foodborne illnesses in the U.S.A. from all food products were 16,614 cases, comprising 15% of the population (37.4 cases per 100,000) in 2005, with Salmonella, Campylobacter, and Shigella being the most commonly reported pathogens [12]. These incidences are further evidence that new and improved tools need to be developed that can be used to better control foodborne pathogens. Currently, aseptic handling to prevent entry of microorganisms into food, mechanical removal of microorganisms (washing or filtration), destroying microorganisms (heat, pressure, irradiation, or chemical sanitizers) and inhibiting the growth of food pathogens and spoilage organisms through environmental control are approaches to food preservation [14, 15].

Unfortunately, the list of available antimicrobial compounds approved by the FDA for use in foods is very limited (Table 1), and regulatory hurdles make it unlikely that a large number of new food antimicrobials will be approved for use in foods in the near future. It is thus imperative to make better use of available approved antimicrobial compounds. Food scientists have thus focused on the development of nanoencapsulation technologies to help improve the functionality of approved food antimicrobials. As with most of the other bioactive compounds (e.g., flavors, antioxidants, and nutraceuticals), antimicrobial agents are chemically diverse [14]. This raises considerable problems when attempting to introduce these compounds into a complex food system. For example, the addition of bioactive compounds may negatively affect the physical stability of the food system and alter the chemical integrity and biological activity of the bioactive compounds [16]. For food antimicrobials, the consequence of low activity is that high concentrations of antimicrobials must be used to effectively control the growth of microorganisms [17].

In order to inhibit the growth of microorganisms, antimicrobial compounds must directly interact with the target organisms, and partitioned into either the microbial membranes or the microbial intracellular space. Physical and chemical processes can alter the structure and functionality of antimicrobials, thereby preventing the interaction of antimicrobials with target pathogens or spoilage organisms [18]. Ingredient interactions may thus have a profoundly negative impact on the ability of antimicrobials to successfully disrupt membrane integrity [19].

This review article provides the necessary background and key physical concepts that will enable a wide range of interdisciplinary researchers to enter the important field of antimicrobial nanoemulsions. Encapsulation of antimicrobial compounds has been shown to reduce ingredient interactions and allow for better control over mass transport phenomena and chemical reactions. Moreover, encapsulation of antimicrobial compounds may increase the effectiveness of concentration in areas of the food system where target microorganisms are preferentially located (e.g., in water-rich phases or at solid–liquid interfaces) [16, 20].

3. Methods of nanoemulsions preparation

Nanoemulsions are non-equilibrium systems of structured liquids with a range of very small particle sizes, so their preparation involves the input of a large amount of either energy or surfactants, and in some cases, a combination of both [6, 21]. They can be most effectively produced using a mechanical, device known as a homogenizer (eg. high-shear mi-

Table 1. List some GRAS antimicrobial agents for use in foods.

Compound	Microbial target	Food application	Reference
Acetic acid, acetates, diacetates, dehydroacetic acid	Yeasts, bacteria	Baked goods condiments, confections dairy products, fats and oils meats, sauces	54
Benzoic acid, benzoates	Yeasts, molds	Beverages, fruit products margarine	54, 55
Dimethyl dicarbonate	Yeasts	Beverages	56
Lactic acid, lactates	Bacteria	Meats, fermented foods	54
Lactoferrin	Bacteria	Meats	57
Lysozyme	*Clostridium botulinum*, other bacteria	Cheese, casings for frankfurters, cooked meat and poultry products	58, 59
Natamycin	Molds	Cheese	60
Nisin	*C. botulinum*, other bacteria	Cheese, casings for frankfurters, cooked meat and poultry products	17
Nitrite, nitrate	*C. botulinum*	Cured meats	56
Parabens	Yeasts, molds	Beverages, baked goods syrups, dry sausage	47
Propionic acid, propionates	Molds	Bakery products, dairy products	56, 61
Sorbic acid, sorbates	Yeasts, molds and bacteria	Most foods, beverages wines	62, 63
Sulfites	Yeasts, molds	Fruits, fruit products, potato products, wines	56, 64

xer, high-pressure homogenizer, colloid mill sonicator or membrane homogenizer) [12,22-23]. The most commonly used methods for producing nanoemulsions are high-pressure homogenization and microfluidization, which can be used at both laboratory and industrial scales (Figure 1). Other methods like ultrasonicfication and in-situ emulsification are also suitable but are mostly used at laboratory scale and not for commercial production [24].

3.1. High pressure homogenization

This technique makes use of a high-pressure homogenizer to produce nanoemulsions of extremely low particle size (up to 1nm). In a high-pressure homogenizer, the dispersion of two liquids (oily phase and aqueous phase) is achieved by forcing their mixture through a small inlet orifice at very high pressure (500 to 5000 psi), which subjects the product to intense turbulence and hydraulic shear resulting in extremely fine particles of

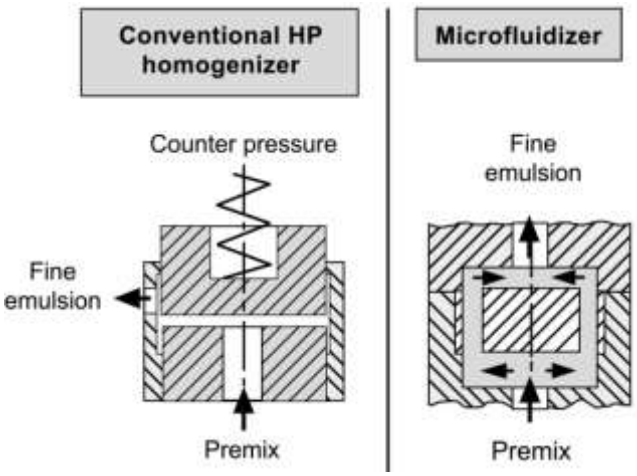

Figure 1. Schematic drawing of the generalized design of a conventional high- pressure homogenization valve (left) and a microfluidizer (right) [26].

emulsion. Homogenizers of varying designs are available for the laboratory and industrial scale production of nanoemulsions. This technique has great efficiency, with the only disadvantages high energy consumption and increase in temperature of emulsion during processing. The two solutions (aqueous phase and oily phase) are combined together and processed in an inline homogenizer to yield a coarse emulsion. The coarse emulsion is then fed into a microfluidizer where it is further processed to obtain a stable nanoemulsion. The coarse emulsion is passed through the interaction chamber of the microfluidizer repeatedly until the desired particle size is obtained. The bulk emulsion is then filtered through a filter under nitrogen to remove large droplets, resulting in a uniform nanoemulsion [5, 25].

3.2. Microfluidization

Microfluidization is a patented mixing technology, which makes use of a device called a microfluidizer. This device uses a high-pressure positive displacement pump (500 to 20000 psi), which forces the product through the interaction chamber, consisting of small channels, called microchannels. The product flows through the microchannels on to an impingement area resulting in very fine particles of submicron range. As the microfluidizer has no moving parts, maintenance is typically lower. Concerning the negative side, there is an increased risk of blockage at the exit of the homogenization chamber, which may be quite difficult to resolve [5].

4. Functional properties of nanoemulsions

Nanoemulsions have some interesting functional properties such as appearance, physical and chemical stability, texture, and activity of encapsulated bioactive compounds that distinguish them from ordinary microscale emulsions [26]. We do not intend to provide a comprehensive review of all the possible properties; rather these particular properties serve as a few primary examples. Different characterization parameters for nanoemulsions are discussed in the following sections.

4.1. Appearance

Nanoemulsions appear visibly different from microscale emulsions since the droplets can be much smaller than optical wavelengths of the visible spectrum, so most nanoemulsions appear optically transparent. Consequently, the appearance of emulsions is strongly dependent on droplet size, and the emulsions become transparent when the size of the droplet falls below a critical diameter (d<90–100 nm). Nanoemulsions are thus easily distinguishable from conventional emulsions and may be quite attractive to the beverage manufacturers trying to avoid the introduction of turbidity with the addition of antimicrobial carrying emulsions [24, 25-26].

4.2. Gravitational stability

The stability of emulsions to gravitational separation (creaming or sedimentation) increases as the droplet size decreases, with creaming velocity proportional to d^2. When the droplet size falls below a critical value (d~100 nm), emulsions become completely stable to creaming or sedimentation because the effects of Brownian motion dominate the gravitational effects. Nanoemulsions can thus be kinetically stable for many years, a property that makes them again very attractive to food manufacturers [26].

4.3. Aggregation stability and rheology

Some mechanical shear or rheological properties of nanoemulsions are affected by droplet size, particularly when the size becomes small. As with microscale emulsions the rheological properties depend strongly on whether the droplets interact repulsively or attractively [27]. The magnitude of repulsive interactions (e.g., steric and electrostatic) and attractive interactions (e.g., Van der waals and depletion) tend to increase with the decrease of droplet size. Droplet collision frequency tends to

increase with the decrease of droplet size, which may promote droplet aggregation [28]. For example, a significant increase in viscosity may be observed in emulsions stabilized by ionic surfactants when the particle size is reduced because this alters the ratio between the thickness of the Debye layer and the droplet diameter, causing a virtual increase in droplet concentration [2].

4.4. Ostwald ripening

Ostwald ripening is a mechanism whereby the larger droplets grow at the expense of the smaller ones because of the molecular diffusion of the oil between the droplets through the continuous phase. This process is driven by the Kelvin effect where the small lipid droplets have higher local oil solubility than the larger droplets because of the difference in Laplace pressure. Coalescence phenomena due to Ostwald ripening can affect nanoemulsion stability, leading to a significant growth in droplet size over time [8, 29-30]. The rate of Ostwald ripening in nanoemulsions depends on the aqueous solubility of the oil, and it has recently been pointed out that Ostwald ripening may possibly be used as a tool in the determination of the solubility of oils in water [8, 31]. Lipids that have appreciable water solubility (e.g. low molecular weight lipids) are thus difficult to incorporate in nanoemulsions. With these compounds, nanoemulsions must be formulated with highly insoluble carrier oil, mixed with the more water soluble antimicrobial [8].

4.5. Bioactive exposure

The surface area of lipid phase at oil-water interface increases with decreasing the particle size, which may impact the chemical stability and bioavailability of lipophilic components. At a constant lipid volume fraction, the surface area is inversely proportional to the mean particle diameter. If the chemical degradation of a lipophilic functional component (e.g., a chemically susceptible antimicrobial) is catalyzed by an aqueous phase component (e.g., Fe^{2+}), having a greater fraction of the lipophilic component at the interface may be detrimental to stability and activity. On the other hand, having increased concentration of an antimicrobial at the droplet interface could be beneficial since the interaction with microbial surfaces and delivery of the antimicrobial could be enhanced [32, 33].

4.6. Bioactive solubility

The solubility of encapsulated lipid antimicrobials in the surrounding aqueous phase increases with decreasing the particle size due to curvature effect, which again may negatively impact the chemical stability of the antimicrobial but possibly improve bioactivity [28, 34- 35].

5. Advantages of nanoemulsions

The attraction of nanoemulsions for diverse applications is due to the following advantages [35, 36, 37-38]:

(a) Nanoemulsions have a much higher surface area and free energy than macroemulsions, which makes them an effective transport system.

(b) Nanoemulsions do not show the problems of inherent creaming, flocculation, coalescence and sedimentation, which are commonly associated with macroemulsions.

(c) Nanoemulsions can be formulated in a variety of formulations such as foams, creams, liquids, and sprays.

(d) Nanoemulsions are non-toxic and non-irritant; hence, they can be easily applied to skin and mucous membranes.

(e) Since nanoemulsions are formulated with surfactants, which are approved for human consumption (GRAS), they can be taken by the enteric route.

(f) Nanoemulsions do not damage healthy human and animal cells, and are, hence, suitable for human and veterinary therapeutic purposes.

6. Disadvantages of nanoemulsions

In spite of the above advantages, nanoemulsions have some disadvantages for the following reasons [25, 37-39]:

(a) Use of a large concentration of surfactant and co-surfactant necessary for stabilizing the nano droplets.

(b) Instability can be caused due to the Oswald ripening effect.

(c) Having limited solubility capacity for high melting substances.

(d) The surfactant must be non-toxic for using pharmaceutical applications.

(e) Nanoemulsion stability is influenced by the environmental parameters such as temperature and pH.

7. Applications of nanoemulsions

7.1. Use of nanoemulsions in cosmetics

Nanoemulsions have recently become increasingly important as potential vehicles for the controlled delivery of cosmetics and for the optimized dispersion of active ingredients, in particular skin layers. Nanoemulsions' lipophilic interior and small sized droplets with high surface areas allow effective transport of active ingredients to the skin. This may reduce the trans-epidermal water loss, indicating that the barrier function of the skin is strengthened [40].

7.2. Prophylactic in bio-terrorism attack

Owing to their antimicrobial activity, research has begun on the use of nanoemulsions as a prophylactic medication (a human protective treatment) to protect people exposed to bio attack

pathogens such as anthrax and Ebola [41]. A broad spectrum nanoemulsion was tested on surfaces by the USA Army in December 1999 for decontamination of anthrax spore surrogates. The technology has been tested on gangrene and *Clostridium botulinum* spores, and can even be used on contaminated wounds to salvage limbs. The nanoemulsion technology can be formulated into a cream, foam, liquid or spray to decontaminate a variety of materials [4,42].

7.3. Nanoemulsions as mucosal vaccines (under trial)

Nanoemulsions are being used to deliver either recombinant proteins or inactivated organisms to a mucosal surface to produce an immune response. The first applications, an influenza vaccine and an HIV vaccine, can proceed to clinical trials. Nano-emulsions cause proteins applied to the mucosal surface to be adjuvanted, and facilitate the uptake by antigen capturing cells, this results in a significant systemic and mucosal immune response that involves the production of specific IgG and IgA antibodies as well as cellular immunity. Research has also demonstrated that animals exposed to recombinant gp120 in nanoemulsions on their nasal mucosa develop significant responses to HIV, thus providing a basis to examine the use of this material as an HIV vaccine. Additional research is ongoing to complete the proof of concept in animal trials for other vaccines including hepatitis B and anthrax [38].

7.4. Nanoemulsion as non-toxic disinfectant cleaner

A breakthrough nontoxic disinfectant cleaner developed by Enviro Systems, Inc., for use in commercial markets, including healthcare, hospitality, travel, food processing, and military applications, kills tuberculosis and a wide spectrum of viruses, bacteria, and fungi in 5 to 10 minutes without any of the hazards posed by other categories of disinfectants [24]. The product needs no warning labels. It does not irritate eyes, and can be absorbed through the skin, inhaled or swallowed without harmful effects. The disinfectant formulation is made up of nanospheres of oil droplets $\leq$ 106 nm, which are suspended in water to create a nanoemulsion requiring only miniscule amounts of the active ingredient, parachlorometaxylenol (PCMX). The nanospheres carry surface charges that efficiently penetrate the surface charges on microorganisms' membranes much like breaking through of an electric fence. Unlike the 'drowning' cells, the formulation allows PCMX to target and penetrate the cell walls. As a result, PCMX is effective at concentrations lower than those of other disinfectants; hence, there are no toxic effects on people, animals, or the environment [43, 44-45].

7.5. Nanoemulsions in cell culture technology

Cell cultures are used for in vitro assays or to produce biological compounds, such as antibodies or recombinant proteins. To optimize cell growth, the culture medium can be supplemented with a number of defined molecules or with blood serum. Up to now, it has been very difficult to supplement the media with oil soluble substances that are available to the cells, and only small amounts of these lipophilic compounds could be absorbed by the cells. Using nanoemulsions is a new method for the delivery of oil soluble substances to mammalian cell cultures. The delivery system is based on a nanoemulsion, which is stabilized by phospholipids. Oily phase uptake of oil soluble supplements in cell cultures, and improved growth and vitality of cultured cells are advantages of using nanoemulsions in cell culture technology [45].

7.6. Application of nanoemulsion in antimicrobial compounds delivery

Nanoemulsions may theoretically be designed in a variety of different ways to serve as carriers of antimicrobials to improve the safety and quality of foods. Antimicrobial nanoemulsions are oil in water droplets (particle size: 20 to 200 nm). They are composed of antimicrobial agents, oil and water, which are stabilized by surfactants and alcohol. Nanoemulsion droplets function by fusing with lipid bilayers of the cell membranes. This fusion is increased by the electrostatic attraction between the anionic charge on the pathogen and the cationic charge of the emulsion; then the energy reserved in the oil and detergent emulsion is released and destabilizes the lipid membrane of the pathogen. Both the active ingredient and the energy released destabilize the pathogen's lipid membrane, resulting in cell lysis and death (Figure 2). The antimicrobial activity of nanoemulsions is nonspecific, unlike that of antibiotics, thus allowing broad spectrum activity while limiting the capacity for generation of resistance. These features make nanoemulsions a suitable candidate for both wound treatment, and surface decontamination [40,41-44].

In the case of spores, additional germination enhancers such as L-alanin, L-proline, Inosine, calcium chloride and ammonium chloride are incorporated into the emulsion. These germination enhancers initiate the germination of spores and the tough outer shells of the spores then become vulnerable to the lethal effect of the nanoemulsion, this results in disruption of the spores (Figure 3). A unique aspect of nanoemulsions is their non-selective toxicity to microbes at concentrations that are non-irritating to the skin or mucous membrane. The safety margin of nanoemulsions is due to the low level of detergent in each droplet, yet when acting in concert, these droplets have sufficient energy and surfactant to destabilize the targeted microbes with-

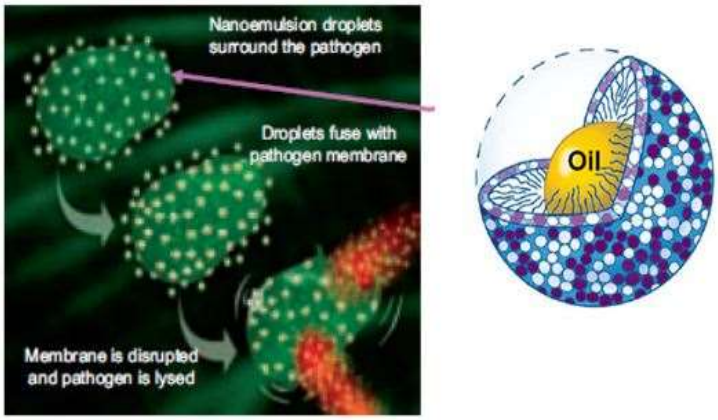

Figure 2. Nanoemulsions kill microbes by a physical mechanism where high energy nanoemulsion droplets fuse with lipid on the outer surface of the pathogen causing physical disruption and lysis of pathogen (left), NEs are composed of nanometer-sized droplets with an oil core stabilized by surfactants (right) [41,65].

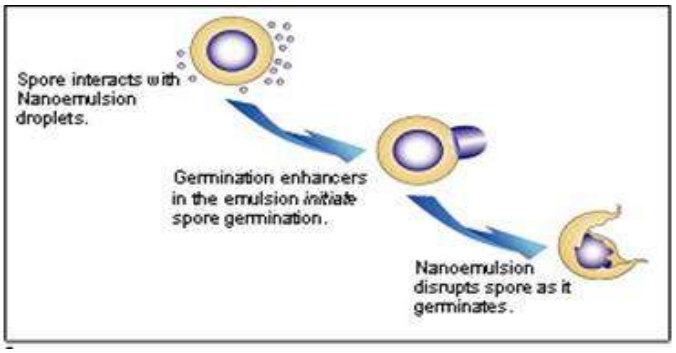

Figure 3. Nanoemulsion mechanism of action against spores [65].

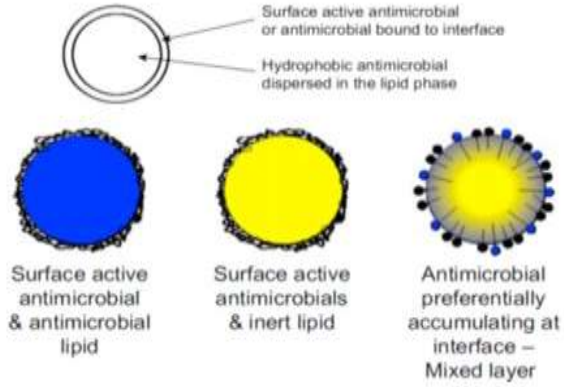

Figure 4. The structures of antimicrobial nanoemulsions. From left to right: nanoemulsions with antimicrobial lipid and surfactant; nanoemulsions with antimicrobial surfactant and inactive lipid; nanoemulsions with preferentially accumulated lipid antimicrobial and inactive surfactant [3].

out damaging the healthy cells. As a result, nanoemulsions can achieve a level of topical antimicrobial activity that has only been previously achieved by systemic antibiotics [46**].**

8. Species of antimicrobial nanoemulsions

Firstly, the lipid phase of nanoemulsions may be loaded with a lipophilic antimicrobial. In this case, the delivery of antimicrobial may occur via a mass transport of the antimicrobial from the inside of the nanoemulsion droplets through the aqueous phase of the food system to the membrane of food pathogens or spoilage organisms (Figure 4). This process would be governed by the solubility of the lipid antimicrobial in the aqueous phase, which is a function of temperature, composition of the aqueous

phase, chemical structure of the antimicrobial and the droplet size. Because of their small size and large curvature, nanoemulsions can be expected to have better activity, since the driving force for the mass transport process (i.e., the concentration difference between the antimicrobial in the vicinity of the oil droplet and in the bulk phase) is much higher due to the Laplace effect [26]. Secondly, antimicrobial nanoemulsions can be constructed from a surface active antimicrobial that stabilizes the nanoemulsion and an inert lipid. Examples of surface active antimicrobials include lysozyme, nisin, and lauric arginate. Additional emulsifiers may be required to enhance emulsion formation and to achieve the small droplet diameter needed to produce nanoemulsions, as well as to improve the stability of nanoemulsions for breakdown of such mechanisms as coalescence or flocculation.

Additional emulsifiers also allow for the interaction of droplets with the microorganisms, which can be improved by creating an electrostatic attraction between the droplets and the negatively charged microbial surfaces.

Thirdly, antimicrobial nanoemulsions could be constructed by combining the two approaches outlined above (i.e., using one antimicrobial that is part of the dispersed droplet phase and a second antimicrobial that is surface active and part of the emulsifier layer). It is to be noted, though, that some antimicrobials (e.g., simple and more complex phytophenols) may not strictly fall into either category. While antimicrobials are typically added to the lipid phase of nanoemulsions, they have a tendency to accumulate in higher concentrations near the vicinity of the interface. In the case of phenolic antimicrobials, this is because the hydroxyl group facilitates an interaction with the headgroup of the surfactant [22,26].

9. Effect of antimicrobial nanoemulsions on various microorganisms

Nanoemulsions have a broad antimicrobial activity against bacteria (e.g., *E. coli*, Salmonella and *S. aureus*), enveloped viruses (e.g., HIV and herpes simplex), fungi (e.g., Candida and Dermatophytes), and spores (e.g., anthrax) at concentrations that are nontoxic in animals [47].
Substantial nanoemulsion formulas for antimicrobial purposes have been made from soybean oil (SBO), which does not possess antimicrobial activity. An SBO-based nanoemulsion named BCTP was produced from SBO, Triton x-100, and tri-n-butyl phosphate in 20% water. BCTP showed an effect by which more than 90% of Bacillus spores could be inactivated within 4 h when they were exposed to this nanoemulsion [48]. BCTP could also inactivate vegetative cells and inhibit the formation of biofilms of Salmonella spp, *E coli* 0157:H7, *Pseudomonas aeruginosa*, and *S. aureus* [47].

For a decade, the essential oils, which are natural preservatives such as lemon grass, lemon [49], oregano [49, 50], rosemary, sage, clove, thyme [11, 49], tea tree, and lemon myrtle oil [51] have been examined against foodborne bacteria in a mixture form. Although, these essential oils provide good antimicrobial properties, excessive amounts used for preserving food may affect their organoleptic property. Lemon myrtle oil, the Australian based plant widely used as an ingredient in many foods, can irritate the human skin at a high concentration [51]. To date, only its minimum inhibitory concentration (MIC) has been reported. Hayes and Markovic studied the effect of lemon myrtle oil against many bacteria including *E. coli*, and found that the MIC for treating *E. coli* was 0.03 % v v^{-1}. Also, nanoemulsions prepared from essential oils to have a stable shelf-life and a synergistic antimicrobial effect have not been studied [1,7].

Many researches focus on the encapsulation of essential oils into nanometric delivery systems for incorporation into fruit juices in order to enhance their antimicrobial activity and improve the safety and quality of foods by the addition of natural preservatives. A mixture of terpenes (from *Melaleuca alternifolia*) and D-limonene was prepared by high pressure homogenization (300 MPa) and encapsulate into food grade nanoemulsions [52]. Lecithin based nanoemulsions with the terpenes mixture were found to be a highly efficient carrier system. D-limonene was successfully nanoencapsulated pure or with palm oil using such emulsifiers as modified starch and soy lecithin. Determining the values of MIC and MBC (minimum bactericidal concentration) in three different classes of microorganisms (*Lactobacillus delbrueckii*, *Saccharomyces cerevisiae*, and *E. coli*) was investigated for antimicrobial activity of terpenes. The MIC values were reduced with nanoencapsulation of D-limonene but they had no significant effects in the MBC values in comparison to the unencapsulated D-limonene. Real systems (i. e. orange and pear juices), inoculated with *L. delbrueckii* and terpens nanocapsules (in concentration of 1.0-5.0 g l^{-1} terpenes) were studied.

Successful microorganism inactivation is dependent on the kind of microorganisms, composition of formulation and droplet diameter. The GC-MS analysis can be used for detecting the chemical stability of active compounds in nanoemulsion [28,47].

Microemulsions and nanoemulsions can be used in biofilm agents for antimicrobial activities [33]. BCTP (O/W including soybean oil and tri-n-butyl phosphate emulsified with triton X-100) and TEOP (O/W including ethyl oleate with tween 80 as emulsifier and n-pentanol as co-emulsifier) as two emulsions that have inhibited some of the famous microorganisms such as Salmonella spp, *E. coli* O157:H7, *P.aeruginosa*, *S. aureus* and *Listeria monocytogenes* [47]. TEOP was effective against all of the mentioned organisms while BCTP was effective only against *L. monocytogenes*. With the

exception of the biofilm formed by *L. monocytogenes*, which surprisingly was not significantly affected by BCTP, the activities and biofilms formed of all the other four bacteria were inhibited by both BCTP and TEOP [27, 47-53].

Many types of bacteria participate in the formation of dental biofilms. More than five Streptococcus species and *Actinomyces viscosus* are regarded as early colonizers of tooth surfaces, whilst streptococci such as *S sobrinus* and *S mutans* are considered late colonizers of the dental biofilms. Synthetic antimicrobials and many of the substances such as povidone iodine products, chlorhexidine, cetylpyridinium chloride, triclosan and zinc citrate have had undesirable effects (vomiting, diarrhea and tooth staining).

Application of nanoemulsions to control the adhesion of biofilm based on these cariogenic bacteria on the tooth surface is a rational approach to prevent this common oral disease. As shown through MIC/MBC assays, nanoemulsions were effective against *S. mutans* at concentrations ranging from 1:100 to 1:10,000, and against planktonic cells at high concentrations. In previous studies 4-day-old *S. mutans* biofilms were treated with nanoemulsions and statistical results showed that subsequent reductions of bacterial cell counts were revealed with decreasing the concentration. Staining of nanoemulsion treated biofilms (with LIVE/DEAD BacLight Bacterial Viability Kit) showed significant increases at all time points in the dead cell counts and areas (up to 48% in 1 min, 84% at 1 h). Destruction of cell membranes and cell walls of *S. mutans* by nanoemulsions was illustrated by scanning electron microscopy [37].

10. Conclusion

This review article highlights the potential of nanoemulsions to efficiently encapsulate and deliver antimicrobial compounds. In conclusion, antimicrobial nanoemulsions are nonphospholipid-based, inexpensive, stable, non-toxic, and non-specific antimicrobial agents that have clinical applications. An efficient design of nanoemulsion-based delivery systems in terms of mean droplet diameters achieved and choice of emulsifier used can affect the possible mechanisms of antimicrobial action of nanoencapsulated antimicrobial compounds. The antimicrobial activity of nanoencapsulated antimicrobial compounds can be carried out through the transport of the antimicrobial compounds across the cellular membrane, where they are released and can act from the inner side on the cytoplasmic membrane, as well as through the controlled release of antimicrobial compounds in the aqueous phase from the nanoemulsion droplets, and therefore, maintaining the essential oils active in the food system over an extended period of time, despite their limited solubility in the aqueous phase. In the future, it seems possible to consider integrating consolidated top-down techniques such as high pressure homogenization with the bottom-up approaches based on thermodynamic self-assembling at molecular levels in order to incorporate complex structures with advanced functionalities in unfavorable environments such as most foods during their manufacturing, storage and preparation. More efforts are also needed to enhance the ability of nanoengineered particles and emulsions to resist the detrimental effects of processing conditions during food production due to the thermolabile nature of bioactive compounds and/or due to the possibility of binding irreversibly to the food structure. Redesign of processing flow sheets and optimization of processing conditions for production of foods supplemented with nanobioactive compounds are also perspectives and challenges in order to prevent the loss of bioavailability.

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Conflict of interests:

The authors declare no conflict of interest**.**

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