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Review

Nanoemulsion-Based Delivery of Essential Oils for Controlling Foodborne Pathogens and Spoilage Microorganisms

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Abstract

The increasing incidence of foodborne diseases and the limitations of conventional preservation methods have driven the search for safer, more effective, and sustainable antimicrobial strategies. In this context, essential oil nanoemulsions have emerged as promising alternatives to synthetic preservatives due to their broad-spectrum antimicrobial activity, natural origin, and potential applicability across diverse food matrices. This study critically examines the mechanisms of action of essential oils against pathogenic and spoilage microorganisms and discusses how their incorporation into nanoemulsions can overcome limitations such as low volatility, poor solubility, and chemical instability. The physicochemical principles governing the formation and stability of these nanoemulsions are addressed, alongside the influence of food matrix components (proteins, lipids, polysaccharides, pH, and ionic strength) on antimicrobial efficacy. Evidence from real food systems indicates that nanoemulsions often outperform free essential oils, although the magnitude of the effect strongly depends on matrix complexity and processing or storage conditions. The review further discusses critical aspects related to toxicity, safety, bioaccessibility, sensory acceptance, and regulatory considerations, as well as emerging evidence on adaptive responses and antimicrobial resistance risks associated with sublethal exposure to essential oil nanoemulsions. It is concluded that, despite their considerable technological potential, the industrial application of essential oil nanoemulsions requires further systematic studies in real foods, standardized protocols, and integrated risk assessments to ensure efficacy and safety under practical conditions.

Keywords: nanotechnology; antimicrobial activity; nanoparticles; food preservative; lipid; essential oils; mechanisms

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1. Introduction

Foodborne pathogens such as *Campylobacter*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella* spp. are frequently associated with outbreaks linked to contaminated meat products, dairy products, ready-to-eat foods, fresh produce, and minimally processed foods [1]. These microorganisms are responsible for approximately 9.9 million cases of foodborne illness annually, resulting in 53.300 hospitalizations and approximately 931 deaths in 2019 [2].

Beyond health impacts, microbial contamination generates substantial economic losses throughout the food supply chain. According to estimates from the Food and Agriculture Organization of the United Nations [3], approximately 1.3 billion tons of food are lost annually worldwide, representing about one-third of all food produced for human consumption. Food losses account for 8–10% of global annual greenhouse gas emissions and may generate economic costs of up to USD 1 trillion per year [4].

In addition to food waste, microbial spoilage is among the main causes of food loss, with microorganisms such as *Pseudomonas* spp., lactic acid bacteria, yeasts, and molds being the most well-known spoilage agents of products such as fresh meats, fruits, dairy products, bakery goods, and beverages.

Socially, the high incidence of microbial contamination undermines consumer confidence in food systems and exacerbates food insecurity, particularly among low-income populations with limited access to safe foods and effective food safety infrastructure. This scenario is especially alarming considering that approximately 783 million people suffer from hunger worldwide each year, and nearly 150 million children under five years of age experience stunting and impaired development due to chronic deficiencies of essential nutrients in their diets [4]. Collectively, these public health, economic, social, and nutritional consequences underscore the urgent need for effective, safe, and sustainable strategies to control pathogenic and spoilage microorganisms in foods.

To mitigate these risks, the food industry relies on conventional preservation strategies such as thermal treatments (pasteurization and sterilization), chemical preservatives, refrigeration, drying, and modified-atmosphere or vacuum packaging. Thermal processing can degrade heat-sensitive nutrients, reduce antioxidant activity, and negatively affect texture, color, and flavor, particularly in fruit-based products, dairy systems, and ready-to-eat foods [5–7]. Chemical preservatives, although efficient, face increasing consumer rejection due to toxicological concerns, allergenicity, and the growing demand for clean-label products [8,9]. Refrigeration and packaging-based strategies may alter sensory attributes or, in some cases, favor the survival of psychrotrophic spoilage microorganisms or anaerobic pathogens, respectively [10–12].

In light of the limitations and drawbacks of traditional food preservation methods, essential oils (EOs) have emerged as promising alternatives to synthetic preservatives [13,14]. EOs from oregano, thyme, clove, cinnamon, rosemary, and lemongrass, rich in compounds such as carvacrol, thymol, eugenol, cinnamaldehyde, and citral, have demonstrated strong antimicrobial activity against major foodborne pathogens and spoilage microorganisms (Table 1). However, their direct application in foods is limited by low water solubility, volatility, chemical instability, strong aroma, and undesirable sensory impacts, particularly in complex food matrices containing proteins, fats, and carbohydrates that can reduce antimicrobial efficacy.

Nanoemulsions (NEs) have therefore emerged as a solution to overcome these limitations and enhance the performance of lipophilic natural antimicrobials in foods. NEs are colloidal systems composed of oil droplets with diameters typically between 20 and 200 nm dispersed in an aqueous phase and stabilized by food-grade surfactants [15–17]. Their small droplet size provides high physical stability, improved solubility, uniform distribution, and increased surface area for interaction with microbial cells [15–17]. They can be incorporated into beverages, dairy products, sauces, emulsified foods, and semi-solid matrices with minimal impact on texture, appearance, and flavor (Table 1).

Despite their promising antimicrobial performance in model systems, the behavior of NEs in complex food matrices, characterized by heterogeneous composition, variable pH, ionic strength, fat content, and processing conditions, remains insufficiently understood.

Interactions with proteins, lipids, and other food constituents can alter droplet stability, release kinetics, and antimicrobial efficacy during storage and processing.

Therefore, the objective of this literature review is to critically analyze the behavior and effectiveness of antimicrobial NEs when applied to real food matrices rather than simplified laboratory systems. The review aims to identify current knowledge gaps, technological challenges, and future perspectives related to NE–food matrix interactions, regulatory considerations, sensory impacts, and scalability, thereby supporting the rational design of effective, safe, and industrially viable NE-based preservation strategies.

Unlike previous reviews that often focus on in vitro antimicrobial efficacy or NE formulation parameters, the present article offers several distinctive contributions: (i) a critical, mechanism-based analysis of EO action, organized into five subsections that integrate recent transcriptomic evidence on adaptive responses; (ii) a systematic evaluation of NE performance in real food matrices, highlighting how proteins, lipids, polysaccharides, pH, and ionic strength modulate antimicrobial efficacy; (iii) an explicit discussion of emerging risks related to antimicrobial resistance and tolerance following sublethal exposure, including identification of five specific knowledge gaps; (iv) an integrated assessment of safety, toxicity, bioavailability, and regulatory considerations; and (v) practical recommendations for future research and industrial application.

Table 1. Essential oil (EO) nanoemulsions (NEs): composition, target microorganisms, and antimicrobial efficacy.

Source	Active Compound	Target Microorganism	MIC/MBC	Applied Food	Reference
Cardamom EO and lemon balm EO (*Melissa officinalis*)	1,8-cineole, Citral, Linalool	*L. monocytogenes*	MIC: 6.25 mg/mL MBC: 12.5 mg/mL	Refrigerated turkey meat	[18]
NE (medium chain triacylglycerol)	Mint oil	*Staphylococcus aureus*; *L. monocytogenes*	MIC ~1–2 mg/mL (NE vs. free EO has ~2–5× lower MIC)[2]	-	[15]
NE (alginate + thyme/lemongrass/sage EO)	Thyme, lemongrass, sage oils	*E. coli*	Inhibition via film contact (no specific MIC given)	-	[19]
Self-emulsified triple EO NE (sunflower lecithin + Tween 20)	Cinnamon + lemongrass + oregano EOs	*S. aureus*	MIC ~0.5–1.0 mg/mL; MBC ~1.0–2.0 mg/mL	Milk (whole milk 3.3%)	[20]
SMEO NE	Spanish marjoram EO	*L. monocytogenes*; *E. coli* O157:H7	MIC/MBC 0.78–1.56 mg/mL	Spinach leaves	[21]

EO: essential oil; NE: nanoemulsion; MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration.

2. Action of Essential Oils Against Pathogenic and Spoilage Microorganisms

EO exerts multi-target antimicrobial activity via four primary mechanisms—membrane disruption, energy impairment, oxidative stress, and systemic inhibition of virulence factors—as summarized in Figures 1 and 2.

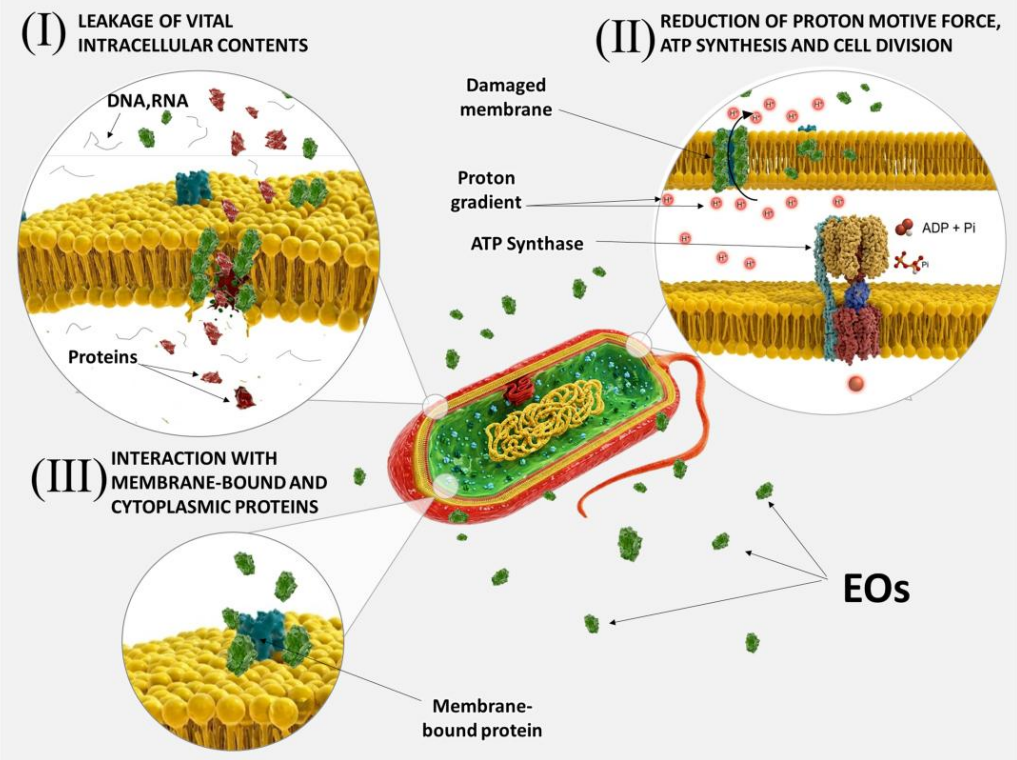

Figure 1. Mechanistic overview of the membrane-targeting action of essential oils (EOs) against microbial cells. The schematic illustrates the main pathways by which EO disrupt cellular structure, bioenergetics, and metabolic functions, ultimately leading to growth inhibition or cell death. (**I**) Membrane disruption and leakage of intracellular contents: Hydrophobic constituents of EO partition into the lipid bilayer, disturbing membrane organization and fluidity. This results in increased permeability and loss of selective barrier function, promoting the leakage of essential intracellular components such as nucleic acids (DNA and RNA), proteins, and ions, which are critical for cellular homeostasis. (**II**) Impairment of cellular energetics: EOs dissipate the proton motive force (PMF) by collapsing both the transmembrane proton gradient (ΔpH) and membrane potential (ΔΨ). This effect may involve proton shuttling or ion-carrying cycles across the membrane. As a consequence, ATP synthase activity is compromised, leading to reduced ATP generation and inhibition of energy-dependent processes, including nutrient transport, biosynthesis, and cell division. (**III**) Protein dysfunction and oxidative stress: EOs interact with membrane-bound and cytoplasmic proteins, altering their structure and function, which results in enzyme inhibition and metabolic imbalance. Additionally, these interactions promote the accumulation of reactive oxygen species (ROS), triggering oxidative stress, lipid peroxidation, and damage to proteins, lipids, and nucleic acids. Collectively, these multi-target effects highlight the broad-spectrum antimicrobial potential of essential oils and their ability to interfere simultaneously with structural integrity, energy metabolism, and redox homeostasis in microbial cells.

2.1. Membrane Disruption and Permeability Alterations

The primary target of EOs is the microbial cell membrane (Figure 1). Hydrophobic components integrate into the lipid bilayer, increasing permeability and causing leakage of intracellular contents. For example, camphor EO caused severe membrane damage in *E. coli* and *S. aureus*, as evidenced by zeta potential shifts (from −25.58 mV to −3.46 mV and from −24.63 mV to −5.42 mV, respectively) and increased leakage of nucleic acids and proteins [22]. Basil EO increased disorder of phospholipid tails in *L. monocytogenes*, leading to leakage of proteins and DNA [23], a mechanism consistent with membrane-disruptive effects reported for EO in other studies [19,24,25]. Carvacrol showed preferential affinity for phosphatidylglycerol and cardiolipins in *Streptococcus pyogenes*, causing membrane depolarization [26]. Other EO, such as those from *Artemisia vulgaris* and *Ocimum basilicum*,

have also demonstrated potent antimicrobial activity, although their specific membrane-disruptive mechanisms require further investigation [27,28].

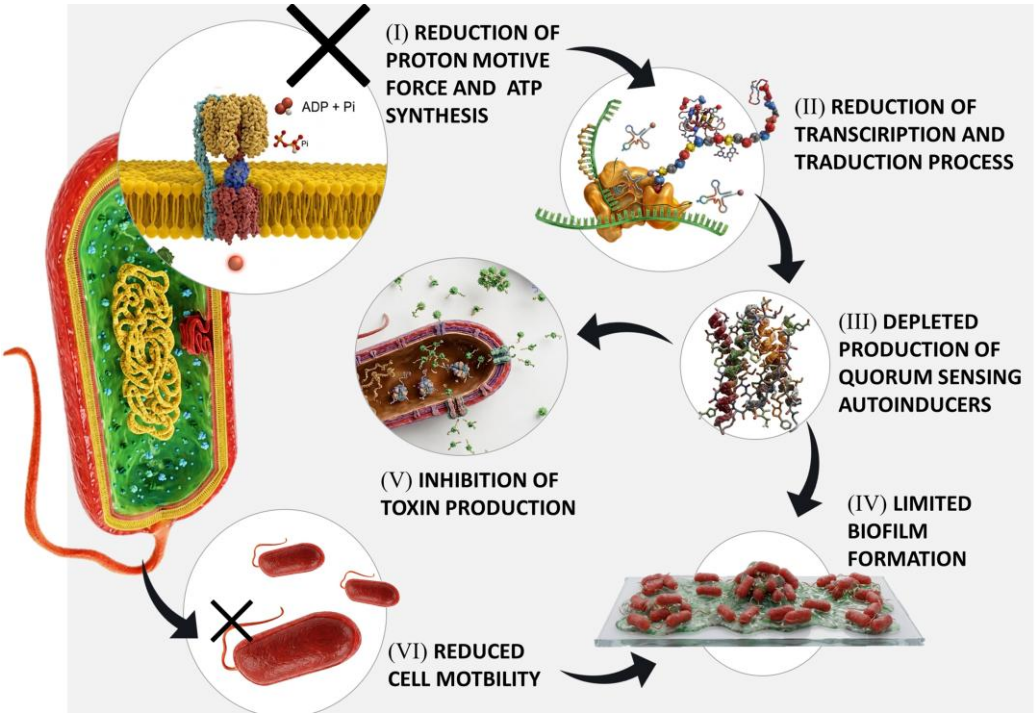

Figure 2. Mechanism of action of essential oils on bacterial cells, highlighting bioenergetic effects and their physiological and regulatory consequences. (**I**) The interaction of hydrophobic compounds from essential oils with the cytoplasmic membrane leads to structural disruption and increased permeability, resulting in dissipation of the proton motive force (PMF) and impairment of ATP synthesis. (**II**) As a consequence of intracellular energy depletion, essential ATP-dependent processes such as transcription and translation are significantly reduced, affecting RNA and protein synthesis. (**III**) The production of quorum-sensing autoinducers is impaired, since their biosynthesis depends on metabolic pathways, gene transcription, and protein translation, leading to reduced cell-to-cell communication. (**IV**) Biofilm formation, which is regulated by quorum-sensing mechanisms, is consequently limited, compromising adhesion, maturation, and stability of these microbial structures. (**V**) The production of enterotoxins, often regulated by quorum sensing and dependent on cellular energy, is inhibited, reducing the virulence potential of microorganisms. (**VI**) Cellular motility, particularly flagellar movement driven by the proton motive force, is impaired, which affects not only bacterial dispersal but also early stages of biofilm formation.

2.2. Impairment of Energy Metabolism and Proton Motive Force (PMF)

EOs can dissipate the PMF and inhibit key metabolic enzymes, compromising cellular energy generation. In *Bacillus cereus*, carvacrol acted as a transmembrane carrier of monovalent cations, cycling between protonated and deprotonated forms. In its undissociated form, carvacrol diffuses across the cytoplasmic membrane into the cytoplasm, where it dissociates and releases a proton. It then reassociates by binding a potassium ion and transports it back across the membrane, ultimately leading to proton influx and potassium efflux [29].

In *L. monocytogenes*, basil EO inhibited the Embden–Meyerhof–Parnas (EMP) pathway via hydrophobic interactions between α-bergamotene and key glycolytic enzymes, such as phosphofructokinase and pyruvate kinase, leading to reduced enzymatic activity and disruption of cellular energy production [30].

Transcriptomic analysis of *Yersinia enterocolitica* exposed to oregano EO revealed that stress induced by the EO led to dissipation of the PMF, triggering activation of the phage

shock protein response and repression of energy-demanding processes such as motility, flagellar assembly, and quorum sensing as an energy-conservation strategy. Concurrently, the upregulation of genes involved in glycerol-3-phosphate metabolism and activation of the PstSCAB transporter contributed to maintaining membrane integrity and energy supply [23].

Furthermore, exposure of *L. monocytogenes* to thymol and cinnamaldehyde resulted in differential expression of 1303 genes, indicating profound metabolic reprogramming. The combined treatment inhibited the biosynthesis of pyrimidines and branched-chain amino acids while activating alternative energy-generating pathways, including the phosphotransferase system (PTS), glycolysis, glycerol catabolism, and the pentose phosphate pathway [31].

2.3. Oxidative Stress Induction

Membrane destabilization and metabolic impairment lead to the accumulation of reactive oxygen species (ROS), triggering lipid peroxidation and protein dysfunction (Figure 1). The combined effects of membrane destabilization and impaired energy production ultimately disrupt bacterial redox homeostasis, promoting excessive accumulation of ROS and triggering widespread oxidative damage [22,30,32].

In bacterial and/or fungal cells, the induction of oxidative stress has been reported for clove (*Syzygium aromaticum*) and *Elsholtzia ciliata* EO [33,34]. Carvacrol-induced ROS accumulation further compromises bacterial redox homeostasis, and the resulting oxidative damage contributes to cell death [35].

As an adaptive response, some microorganisms enhance their antioxidant defenses. For instance, *Y. enterocolitica* exposed to oregano EO increased the expression of genes associated with glutathione peroxidase and amino acid biosynthesis, thereby mitigating oxidative stress-induced damage [23]. Similarly, *L. monocytogenes* exposed to basil EO showed disrupted redox homeostasis, contributing to the overall bactericidal effect [30].

2.4. Inhibition of Cell Division, Virulence, and Biofilm Formation

Sublethal concentrations of EO interfere with cell division and virulence. Carvacrol induced cell elongation in *Bacillus subtilis* and increased cell size in *S. aureus* by facilitating polymerization of FtsZ, a protein crucial for the regulation of bacterial cell division, potentially interacting with the T7 loop of this protein [36].

In addition to effects on cell division, EOs suppress complex cellular functions. When exposed to sublethal concentrations of *Origanum vulgare* L. EO, *Y. enterocolitica* underwent extensive transcriptomic remodeling, with 300 differentially expressed genes. The stress led to repression of energy-demanding processes such as motility, flagellar assembly, and quorum sensing as an energy-conservation strategy [23].

Thymol and cinnamaldehyde together strongly repressed virulence-associated genes in *L. monocytogenes*, including those involved in flagellar motility, chemotaxis, biofilm formation, internalins, and virulence gene clusters, indicating a marked reduction in pathogenic potential under combined stress [31].

EOs also impair quorum-sensing-mediated communication, enterotoxin production, and biofilm formation (Figure 2). The suppression of these processes occurs because energy-demanding pathways may be impaired or halted when cells are exposed to EOs [19,25]. Phenolic terpenoids such as thymol and monoterpene alcohols such as menthol have been shown to destabilize membrane-bound proteins and interfere with enzymatic systems involved in energy metabolism, further contributing to the inhibition of virulence factors [37–39].

2.5. Differential Susceptibility Among Microbial Groups

Gram-negative bacteria generally exhibit greater intrinsic resistance to EO because of the presence of an outer membrane rich in lipopolysaccharides, which acts as an additional barrier to the diffusion of hydrophobic compounds, as well as the high expression of selective porins and efflux systems that limit intracellular accumulation of these compounds [13,14,19,25].

In contrast, Gram-positive bacteria lack an outer membrane and possess a thick peptidoglycan cell wall that, although structurally robust, is more permeable to hydrophobic molecules, making this group generally more susceptible to EOs [13,14,19,25].

Fungi, in turn, display an intermediate level of resistance, mainly associated with the complexity of the cell wall, composed of chitin, glucans, and mannoproteins, and the presence of ergosterol in the plasma membrane, which influences membrane fluidity and interactions with lipophilic compounds [13,14,19,25]. In fungal cells, compounds such as limonene disrupt membrane-associated proteins, inhibit their synthesis, and interfere with fungal cytokinesis [40].

Collectively, the diverse sources and mechanisms underscore the substantial potential of EOs for food preservation. When incorporated into advanced delivery systems such as NE, these compounds can achieve improved solubility, enhanced stability, controlled release, and superior antimicrobial performance in complex food matrices, thereby supporting their practical implementation in safe, effective, and sustainable food systems.

3. Fundamental Mechanisms Governing the Formation and Stability of Essential Oil Nanoemulsions

EO NEs normally consist of colloidal dispersions composed of oil droplets (~100 nm) dispersed in a continuous aqueous phase [oil-in-water (O/W) emulsion type] [41,42]. The EO can be directly incorporated into the aqueous phase or pre-dispersed in an oil carrier (e.g., medium chain triglyceride oil, corn oil, or soybean oil) to enhance protection and improve its dispersion within the food system (Table 2).

Table 2. Emulsifiers used in oil-in-water (O/W) nanoemulsions (NEs) containing essential oils (EOs).

Emulsifier/System	Type/Composition	Application/Function in O/W EO NE	Key Findings/Use Case	Reference
Tween 80 + Span 80 blend	Non-ionic surfactants	Mixed surfactant system to optimize HLB and stability of EO NE	Blend achieved optimal particle size and stability (HLB ≈ 12)	[43]
Tween 80	Polysorbate 80	Common food-grade emulsifier for EO NE	Used as baseline emulsifier for stability and droplet size	[44]
Pea protein isolate (PPI)	Phospholipid	Natural emulsifier in EO NE	Lecithin comparable to Tween 80 in forming stable O/W NE	[45]
Soybean protein isolate (SPI)	O/W ultra-NE-PPI protein alone (no synthetic surfactant)	Stabilizes oil droplets in continuous phase via HPH	Pea protein enables production of ultra-NEs (<100 nm) with improved stability	[46]
Pea protein isolate (PPI)	O/W NE-PPI particles + phycocyanin co-emulsifier	Serve as biological emulsifier/stabilizer at oil–water interface; forms droplets by adsorption	PPI stabilized O/W NE of hempseed oil with small droplet size (~<210 nm) and high entrapment efficiency; lecithin enhances stability	[47]

Table 2. *Cont.*

Emulsifier/System	Type/Composition	Application/Function in O/W EO NE	Key Findings/Use Case	Reference
Pectina + Tween 80	O/W NE-Orange EO + pectina (2–6%) + Tween 80	Pectin acts as natural stabilizer, increasing continuous-phase viscosity and contributing to droplet stabilization	Higher pectin concentration led to increased viscosity and reduced droplet size, improving physical stability of NE	[48]
High-methoxyl pectin (HMP) + Tween 80	O/W NE Lemongrass, mandarin, oregano or thyme EO + HMP + Tween 80	Anionic pectin adsorbs at oil–water interface, contributing electrostatic and steric stabilization	Stable NE (<50 nm for some EO) maintained for up to 56 days, depending on EO type	[49]

NE: nanoemulsion; EO: essential oil; (O/W): oil-in-water.

The theoretical basis of macroemulsion formation was established by classical work of Taylor (1932) [50], who described a fundamental framework linking fluid flow conditions to droplet size. Through combined experimental and theoretical analyses of a single droplet subjected to a laminar flow field, Taylor demonstrated that droplet breakup occurs only when the externally applied hydrodynamic stress exceeds the interfacial stress that maintains droplet integrity. Based on this balance of forces, Taylor introduced the concept of a critical capillary number (C_1), defined as the ratio between the applied deforming stress and the interfacial stress. Below this critical value, droplets undergo deformation but remain stable and do not break, whereas exceeding C_1 leads to droplet disruption and size reduction.

Mathematically, in low-Reynolds-number (Re) flows, the droplet size (d) of the dispersed phase can be defined as a function of the balance between viscous and interfacial forces:

$$d = C_1 \frac{\sigma}{\mu_c \dot{\gamma}} \quad (1)$$

where σ is the interfacial tension; μ_c is the continuous phase viscosity; and γ is the shear rate in the continuous phase.

Taylor's theory is limited to laminar flow regimes and therefore does not represent the real conditions encountered during emulsion production by ultrasonication or high-pressure homogenization. In turbulent regimes, the most widely used framework for predicting droplet size was proposed by Hinze (1955) [51], who suggested that droplet size is governed by the power density input (ε) and the density (ρ_c) of the continuous phase, respectively:

$$d = C_2 \left(\frac{\sigma}{\rho_c} \right)^{3/5} \varepsilon^{-2/5} \quad (2)$$

where C_2 represents a constant similar to Taylor's approach.

Assuming typical NE parameters, droplet diameter $d \approx 100$ nm, dispersed-phase viscosity $\mu_d \approx 10$ cP, dispersed-phase density $\rho d \approx 1000$ kg m^{-3}, and interfacial tension $\sigma \approx 10$ mN m^{-1}, Gupta et al. (2016) [52] proposed that, prior to droplet breakup, a filament is extruded from the parent droplet as a result of a surface instability. Based on Equation (3), the authors mathematically described the characteristic droplet size in NE under the viscous turbulent regime, characterized by turbulent flow in the bulk phase and viscous-dominated flow in the vicinity of the droplets:

$$d = C_3 \frac{\sigma^{5/6} \mu_d^{1/3}}{(\rho_d \sigma)^{1/5} (\mu_c \rho_c \varepsilon)^{5/12}} \quad (3)$$

where C_3 is a constant.

Based on Equation (3), it is possible to predict the droplet size in NE, which is directly governed by the viscosity of the dispersed phase ($\mu_d{}^{1/3}$) and inversely proportional to both the viscosity of the continuous phase ($\mu_c{}^{5/12}$) and the power density input ($\varepsilon^{5/12}$).

From a physicochemical standpoint, the emulsification energy, or Gibbs free energy (ΔG), represents the amount of energy required to disperse one liquid into another immiscible liquid, thereby forming an emulsion. In simplified terms, the free energy of emulsification can be mathematically expressed as the product of the interfacial tension (σ) and the increase in interfacial area (ΔA), such that $\Delta G = \sigma\ \Delta A$. Although this expression neglects the entropic contribution ($T\Delta S$), it is commonly used as a first approximation for conventional emulsions, where the interfacial term predominates.

If $\Delta G > 0$, it can be inferred that the formed system is thermodynamically unstable. In practice, it means that the system spontaneously tends to reduce its free energy by returning to the separation of the immiscible phases, such as oil and water, which corresponds to the thermodynamically more stable condition.

Considering that NEs contain oil droplets with diameters on the order of 100 nm dispersed in an aqueous phase, emulsification leads to a marked increase in interfacial area (more than 10^4–10^6 times) [15,16]. Because the change in free energy is proportional to the increase in interfacial area (ΔA), this enlargement results in positive ΔG values, indicating thermodynamic instability. Although this instability cannot be eliminated, it can be mitigated by lowering the interfacial energy. In this context, surfactants such as Tween 80 (polysorbate 80), Span 80 (sorbitan monooleate), and lecithin have been widely used in the formulation of EO-based NE (Table 2).

The selection of a surfactant is generally based on the hydrophilic–lipophilic balance (HLB), as summarized in Table 2 [15,16].

Still from a thermodynamic perspective, the stability of NE is strongly influenced by Laplace pressure (ΔP), which describes the pressure difference between the inside and outside of a spherical droplet:

$$\boldsymbol{\Delta P = 2\sigma / r} \tag{4}$$

where r is the droplet radius.

Based on Equation (4), as droplet size decreases to the nanoscale, the Laplace pressure increases substantially. Consequently, smaller droplets tend to merge with larger ones, or dissolve and redeposit onto them, in a process known as Ostwald ripening, in order to reduce the internal pressure of the system. Once again, this highlights the fundamental role of surfactants in NE formulation, as they are responsible for reducing interfacial tension and, consequently, the Laplace pressure.

Regarding production methods, high-energy techniques such as high-pressure homogenization, microfluidization, and ultrasonication involve the application of intense mechanical forces, including shear, turbulence, and cavitation, to disrupt micrometric droplets into the nanometric range [53]. These approaches are widely employed in industrial settings due to their high reproducibility and precise control over droplet size distribution. In contrast, low-energy methods, including spontaneous emulsification and phase inversion processes triggered by temperature or compositional changes, rely on physicochemical transformations within the system, particularly reductions in interfacial tension and surfactant molecular rearrangement, to facilitate NE formation with lower energy input [54]. Considering the extensive and well-established literature available on NE production methods, readers are referred to review articles for further details [16,53,54].

4. Impact of Food Matrix Components on the Stability and Antimicrobial Performance of Essential Oil Nanoemulsions

As illustrated in Figure 3, food matrix components such as proteins, lipids, polysaccharides, pH, and ionic strength can either enhance or compromise the stability and antimicrobial efficacy of EO NEs.

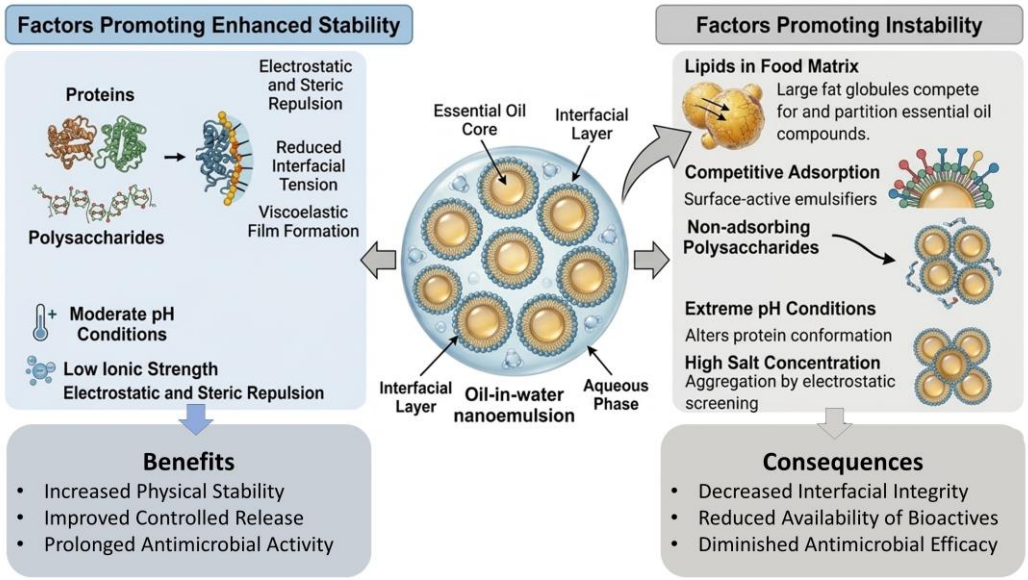

Figure 3. Conceptual representation of how food matrix components modulate the physicochemical stability and antimicrobial performance of essential oil nanoemulsion. Proteins and polysaccharides may enhance interfacial stabilization and controlled release, whereas lipids, extreme pH, and high ionic strength may promote destabilization and reduce bioactive availability.

NEs containing EOs have demonstrated effective control of pathogenic and spoilage microorganisms in various food matrices, including meat products, dairy products, beverages, and minimally processed fruits (Table 2). Due to their nanometric scale, EO-NE increase the surface area available for interaction with microorganisms and enhance contact with microbial cell membranes, creating a microenvironment favorable for antimicrobial actions [55,56]. Therefore, EO NEs may exhibit greater antimicrobial activity than pure (non-emulsified) essential oils in food systems [57].

In turkey meat inoculated with *L. monocytogenes*, the NE containing cardamom and lemon balm EO demonstrated greater antibacterial activity compared to the control (non-emulsified oils), with MIC and MBC values of 6.25 mg/mL and 12.5 mg/mL, respectively [18]. Furthermore, the study revealed that the NE increased nucleic acid leakage by 12.4-fold and protein release by 7.4-fold, and promoted an increase in the extracellular potassium ion concentration from 1.4 to 4.1 ppm compared to the control.

To assess antibacterial activity in a food matrix, the inhibition of *B. cereus* in whole milk by a carvacrol NE at 1 × MIC (0.15 mg/mL) was evaluated using a time–kill assay, with free carvacrol at the same concentration as the control [58]. The NE reduced viable counts from 6 log CFU/mL to undetectable levels within 24 h, whereas free carvacrol allowed growth from 6 to 7 log CFU/mL. The authors attributed the superior efficacy of the NE to improved dispersion and sustained release, which enabled prolonged antimicrobial activity compared to free carvacrol.

The transition from simplified model systems to real food matrices introduces a level of complexity that directly impacts the functionality of NEs. The heterogeneous nature of foods, composed of a network of proteins, lipids, carbohydrates, and minerals, creates

a physicochemical environment that alters the mobility and stability of nanostructured droplets. This complexity often results in lower antimicrobial efficacy than that observed in vitro, requiring a detailed understanding of the molecular interactions involved [59].

Food proteins, including plant-derived proteins from soy and pea as well as animal proteins such as whey and egg proteins, are widely recognized for their surface-active properties and are frequently employed as emulsifiers in the formulation of NEs (Table 2). Owing to the presence of both hydrophilic and hydrophobic amino acid residues, these macromolecules can adsorb at oil–water interfaces, where they reduce interfacial tension and promote the formation of kinetically stable dispersions [60–62].

Nonionic surfactants, such as Tween 80 (polysorbate 80), are electrically neutral and therefore exhibit low sensitivity to pH variations and ionic strength, maintaining stable performance even in the presence of salts [63]. In addition, they show a reduced tendency for undesirable electrostatic interactions with proteins or other charged components, thereby minimizing the risk of precipitation and enhancing system predictability [64].

On the other hand, proteins (soy and pea) and carbohydrates (gum arabic and pectin), when used as emulsifying agents, tend to have greater regulatory and consumer acceptance (Table 2). Proteins adsorb at the oil–water interface and form thick, viscoelastic interfacial films, providing increased resistance to droplet coalescence [65]. Carbohydrates, particularly polysaccharides, contribute mainly through steric stabilization and by increasing the viscosity of the continuous phase, thereby reducing droplet mobility and, consequently, phenomena such as creaming and sedimentation [66]. In some cases, protein-polysaccharide conjugates combine electrostatic and steric stabilization mechanisms, resulting in enhanced overall stability [66–68].

Beyond the selection of emulsifiers, their functional properties can be enhanced through physical and chemical modifications. Yang et al. (2023) [69] demonstrated that ultrasound coupled with weak alkali cycling (from pH 7 to pH 10 and back to pH 7) regulates the interfacial flexibility of flaxseed protein isolates by promoting the exchange between free sulfhydryl groups (-SH) and disulfide bonds (-S-S-). This treatment induced protein deaggregation and reassembly, exposing hydrophobic groups and increasing surface hydrophobicity, which resulted in faster adsorption at the oil–water interface and the formation of a viscoelastic interfacial film with high mechanical stability, ultimately improving emulsification stability by 1.74 times [69]. These findings highlight that protein engineering strategies can expand the applicability of plant-based emulsifiers in NE systems.

In this context, alternative plant proteins have recently been explored for NE stabilization, as illustrated by the following examples. Neto et al. (2024) [70] developed a potato protein-based NE of oregano EO with nanometric sizes (<300 nm) and demonstrated enhanced anti-*E. coli* O157:H7 activity, achieving a 7 log CFU/mL reduction at low concentrations [70].

The interfacial behavior of plant proteins is fundamentally determined by their molecular structure. Yang et al. (2026) [71] investigated the oil–water interfacial adsorption of flaxseed protein isolates and demonstrated that globulin, due to its higher hydrophobicity and viscoelastic modulus, forms a rigid, solid-like interfacial layer that enhances emulsion stability, whereas albumin, with its lower molecular weight and higher solubility, forms a fragile interfacial film that correlates with reduced emulsifying activity [71]. Within the mixed protein system, globulin serves as the primary structural framework while albumin acts as a filler, providing a correlation between protein structure and emulsion stability. These findings highlight that the relative composition of protein fractions determines the performance of plant-based emulsifiers in NE systems.

Although no direct evidence was identified indicating that proteins naturally present in food matrices inherently destabilize nanoemulsified EOs, protein–interface interactions

are highly system-dependent. Depending on environmental conditions and formulation parameters, proteins may enhance NE stability by forming viscoelastic interfacial films or, alternatively, contribute to destabilization phenomena such as flocculation, bridging, or competitive displacement of low-molecular-weight surfactants [72]. For example, in a study involving W/O emulsions stabilized by diacylglycerol-based solid lipid nanoparticles, Chen et al. (2023) [73] reported intense competition between sodium caseinate and the nanoparticles for interfacial adsorption, leading to emulsion destabilization.

It is worth emphasizing that protein adsorption and interfacial behavior are strongly influenced by factors such as pH, concentration and heating. Yang et al. (2023) [74] demonstrated that pH-induced conformational changes in egg yolk proteins significantly affected their functional properties. At pH 9.0, proteins exhibited more unfolded and flexible secondary and tertiary structures, resulting in reduced surface tension, increased solubility, and enhanced emulsifying capacity. In contrast, higher levels of interfacial protein adsorption were observed at pH 3.0, leading to the formation of a thicker interfacial layer predominantly stabilized by electrostatic repulsion between similarly charged proteins at the droplet surface. The authors concluded that, at alkaline pH, the greater solubility and conformational flexibility of non-adsorbed proteins promoted their dispersion in the aqueous phase, contributing to steric stabilization mechanisms.

Zhou et al. (2021) [75] reported that WPI adsorption at sunflower oil–water (o/w) interfaces was faster at higher protein concentrations (up to 0.1 wt%) and at pH 3. Heat-induced surface activity (95 °C for 30 min) was more pronounced at pH 7 than at pH 3, likely due to the greater thermal stability of WPI under acidic conditions. According to the authors, thermal treatment enhanced the elastic behavior of the interfacial layers, as evidenced by longer relaxation times, possibly associated with the formation of intermolecular β-sheet structures.

In fat-rich matrices such as cheese, butter, sausages, and ready-to-eat meat products, endogenous lipids compete with NE droplets for lipophilic antimicrobial compounds. This competition can lead to partitioning of EO components from the NE into the bulk lipid phase, reducing their concentration at the oil–water interface where microbial cells are typically located. Studies on EO NEs incorporated into cheese and meat emulsions have reported reduced antimicrobial efficacy compared to aqueous systems, largely due to lipid–lipid interactions and sequestration effects [76,77]. Furthermore, lipid oxidation and peroxidation processes in the food matrix may propagate into the nanoemulsion oil phase, causing degradation of both carrier lipids and encapsulated bioactives, especially under thermal processing or prolonged refrigerated storage.

Despite the well-established chemical affinity between EO and lipid components of food matrices, no studies were identified that specifically evaluate the influence of lipid concentration on the antimicrobial activity of nanoemulsified EO in real food systems.

For its part, polysaccharides and hydrocolloids, commonly added to foods as thickeners or stabilizers (e.g., xanthan gum, carrageenan, and pectin), influence NE behavior in multiple ways. Increased viscosity of the continuous phase can enhance physical stability by reducing creaming and sedimentation, as predicted by Stokes' law. The addition of guar gum (>0.10% *w*/*w*) to O/W NE increased viscosity while preventing significant changes in the creaming index over 30 days of storage at 5 °C, enhancing the physical stability of the system [78]. In model emulsions rich in polyunsaturated fatty acids (PUFAs), Vilanculos et al. (2023) [79] suggested that chitosan–xanthan interactions promoted steric stabilization. They also argued that the presence of chitosan increased zeta potential values, thereby contributing to enhanced electrostatic stabilization. Recent studies have further demonstrated the potential of polysaccharide-based systems, including chitosan/pullulan coatings with

thyme EO NEs for strawberry preservation [80], alginate-based cinnamon EO NEs [81], and Salecan β-glucan films with lemon EO NEs for chilled pork [82].

On the other hand, non-adsorbing polysaccharides may induce depletion flocculation, particularly in beverage emulsions and sauces, leading to droplet clustering and loss of uniform dispersion [59,83]. Experimental evidence provided by Vilanculos et al. (2023) [79] demonstrated that higher proportions of xanthan gum in NEs can lead to kinetic instability, favoring Ostwald ripening and coalescence.

The impact of pH and salinity further modulates these effects. Acidic environments, such as those found in fruit-based products or fermented foods, can destabilize protein-stabilized NEs, while high salt concentrations in processed meats or brined products compress the electrical double layer around droplets, promoting aggregation through electrostatic screening [59,83,84].

In a flaxseed oil NE model stabilized with flaxseed gum, particle size and polydispersity index remained unchanged between pH 5 and pH 7 [85]. However, both parameters increased at pH 9, likely due to droplet aggregation. The creaming index varied under different pH conditions, following the order pH 7 > pH 5 > pH 3 > pH 9 > pH 11. The authors suggested that droplet aggregation occurs when net attractive forces (mainly van der Waals and hydrophobic interactions) overcome repulsive forces (primarily electrostatic and steric), which typically happens when electrostatic repulsion between droplets is weakened, such as under extreme acidic or alkaline conditions.

In the same study, NEs exhibited greater stability when the NaCl concentration was below 200 mmol/L, whereas stability gradually decreased or even collapsed as NaCl concentration increased [85]. This effect was attributed to the adsorption of Na^+ ions onto negatively charged polysaccharide chains (flaxseed gum), leading to charge screening and a reduction in surface charge, thereby weakening electrostatic repulsion between droplets.

This section critically examined the influence of major food matrix components on the physicochemical stability and, consequently, the antimicrobial performance of nanoemulsified EOs. Notably, systematic studies specifically addressing the interactions between free or nanoemulsified EO and isolated food constituents remain limited. As a result, current interpretations are largely extrapolated from studies evaluating NE–biopolymer, NE–lipid, or NE–protein interactions, which provide mechanistic insights but may not fully capture the complexity of real food systems.

While the effects of individual components were discussed separately to elucidate underlying mechanisms, it must be emphasized that, in actual food matrices, proteins, lipids, carbohydrates, minerals, and other constituents interact simultaneously and dynamically. Such multicomponent interactions can lead to synergistic or antagonistic effects, making it inherently difficult to predict NE stability and antimicrobial efficacy solely based on simplified model systems. Therefore, translating findings from controlled experimental models to real food applications requires careful consideration. In the following section, selected studies demonstrating the practical application of nanoemulsified EO in real food systems will be discussed to bridge this gap between mechanistic understanding and applied performance.

5. Evaluation of Antimicrobial Efficacy in Real Food Systems

As demonstrated in Table 3, the incorporation of NE enabled not only a significant reduction or complete inactivation of pathogenic and spoilage microorganisms but also the maintenance and, in some cases, improvement of physicochemical and sensory properties of food products. These effects are mainly attributed to enhanced kinetic stability, increased specific surface area, and improved interaction between nanostructured droplets and microbial surfaces, resulting in greater bioavailability of bioactive compounds [21,40,86,87].

Table 3. Applications of essential oil (EO)-based nanoemulsions (NEs) in food matrices and their antimicrobial and quality effects during storage.

Food Matrix	NE System	Main EO Components (%)	Target Microorganism(s)	Main Observed Effects	Reference
Strawberry	NE containing *Thymus vulgaris* and *Mentha longifolia* EO	Th: thymol (41.49%); Me: 4-methyl-2-(3-methyl-2-butenyl)-furan (67.52%) and piperitenone oxide (12.51%)	*Botrytis cinerea*	Significant delay in yeast and mold growth; reduction in aerobic mesophilic bacteria, spoilage index, and weight loss; maintenance of physicochemical quality, with higher firmness, vitamin C content, total flavonoids, and antioxidant activity during storage.	[88]
Tomato	Carnauba wax– and *Aloe vera*–based NE coating supplemented with cassia and/or Mexican lime EO	Cinnamaldehyde (77.41%) and limonene (65.36%)	*B. cinerea*	Disruption of fungal cell membrane; complete inhibition of *B. cinerea* growth (MIC: 0.0625 µL/mL and 3 µL/mL); improvement in weight loss, color, firmness, and total soluble solids.	[40]
Spinach leaves	*Thymus mastichina* EO NE (5% *w*/*w*) stabilized with Tween 80 (0.5–5% *w*/*w*)	1,8-cineole (69.15%)	*L. monocytogenes* and *E. coli* O157:H7	Concentration-dependent bacterial reductions, 4–8 times higher than crude EO; efficacy comparable or superior to chlorine-based disinfectants without compromising leaf visual quality.	[21]
Trout filet	O/W NE containing EO blend (oregano: 50%, thyme: 40%, lemongrass: 10%)	ORG: carvacrol (70.3%), p-cymene (10.4%), γ-terpinene (4.8%), (E)-β-caryophyllene (4.7%), linalool (2.2%), myrcene (1.6%); thymol (31.2%), carvacrol (25.5%), p-cymene (21.7%), linalool (6%), limonene (3.4%), borneol (3.4%); geranial (45.5%), neral (33.7%), geraniol (4.5%), geranyl acetate (1.5%), citronellal (1.3%), citronellol (1.1%)	*E. coli*, *S. aureus*, and *Salmonella enterica* serotype Enteritidis	2% NE showed the highest antimicrobial efficacy, with reductions of 0.33, 0.20, and 0.73 log CFU/g for *S. Enteritidis*, *E. coli*, and *S. aureus*, respectively.	[89,90]
Beef burgers	Pectin-based NE (0.055%), Tween 80 (1%), chitosan, and cinnamon EO	(E)-cinnamaldehyde (60.58%); benzyl alcohol (27.36%); triacetin (10.68%); ethylbenzene (0.47%)	*Aspergillus flavus*	Marked reduction in mycelial growth and complete inhibition of spore germination at low concentration (156 µL/100 g) after 9 days of storage.	[91]
Mortadella	Double NE containing ajwain and cardamom EOs at different ratios, stabilized with Tween 80 and glycerol	1,8-cineole (42.93%), α-terpinyl acetate (28.62%), β-phellandrene (11.01%), β-myrcene (6.55%), linalool (2.02%); thymol (58.30%), p-cymene (21.11%), γ-terpinene (14.72%), β-pinene (2.16%), α-terpineol (1.26%)	*S. aureus* and *E. coli*	Synergistic effect between EO, enhancing antioxidant and antibacterial activities; higher efficacy against Gram-positive bacteria; significant reduction in total bacterial counts, with best performance at 2–3%.	[92]
Fermented dairy beverage (Doogh)	*Zataria multiflora* EO NEs (7.5% *v*/*v*) stabilized with Tween 80	Carvacrol (56.64%), α-pinene (23.20%), thymol (10.01%), o-cymene (2.87%), γ-terpinene (1.30%)	*Pseudomonas aeruginosa*, *E. coli*, *S. aureus*, and *B. cereus*	Bactericidal effect associated with morphological and cell wall alterations; effective microbial control during storage, maintaining counts below detection limits and comparable to pasteurized control.	[93]

EO: essential oil; MIC: minimum inhibitory concentration; NE: nanoemulsion; O/W: oil-in-water.

Furthermore, NE efficacy is strongly dependent on the food matrix. Their performance in products such as meat, dairy, seafood, vegetables, and beverages may vary according to matrix-specific interactions, EO chemical composition, and applied concentration. This reinforces the need for evaluations under real food system conditions to validate findings obtained from in vitro studies, which may present limitations when translated to industrial-scale applications (Topic 7).

5.1. Fruits and Vegetables

In fresh and minimally processed fruits and vegetables, the application of EO can be achieved through coatings in which the oil is incorporated into a polymeric matrix. This coating may be applied by spraying onto the plant surface or by immersing the product in a polymeric solution containing the EO, ensuring uniform coverage. These coatings act as functional barriers that reduce water evaporation, modulate respiration rates, and contribute to the maintenance of postharvest quality, showing superior performance compared to conventional emulsions [86,94].

Cardamom and ajwain [95], thyme and lemongrass [87,94], clove [87], and oregano [96,97] EO have been widely investigated for the biopreservation of fruits and vegetables. These compounds have been applied as edible coatings [40,86,88,98], spray solutions [98], and sanitizing treatments for minimally processed leafy vegetables [21,99], demonstrating fungicidal and/or bactericidal effectiveness against *B. cinerea* [40,100], *Shigella flexneri* [101], *L. monocytogenes*, *Salmonella*, *S. aureus*, and *E. coli* [21,96,97,99,102].

Félix-Beltrán et al. (2026) [40] reported antifungal activity in tomatoes treated with NE based on carnauba wax and Aloe vera, supplemented with cassia and/or Mexican lime EO. This effect was attributed to reduced droplet size, increased interfacial area, and improved dispersion of bioactive compounds, leading to membrane destabilization and inhibition of spore germination. Chen et al. (2025) [80] developed a chitosan/pullulan composite edible coating containing 2% thyme EO NE (89.56 nm particle size) for strawberry preservation, achieving 54.7% inhibition of *B. cinerea* mycelial growth and extending shelf life for 12 days at 2 °C [80].

In minimally processed leafy vegetables, NEs have demonstrated high sanitizing potential, with performance comparable to or superior to chlorine-based sanitizers. Cardoso et al. (2023) [96] observed a significant reduction in *Salmonella* and *E. coli* (>2 log CFU/g) in lettuce treated with carvacrol-loaded NE, without negative impacts on color and firmness. Similarly, Chen et al. (2021) [37] applied nanoemulsified carvacrol as a washing treatment for lettuce and spinach, achieving greater microbial reduction in *E. coli* (4 log CFU/cm^2) compared to chlorine treatment (2 log CFU/cm^2).

In fruits, antifungal activity against *A. flavus* has been demonstrated in sugar apple treated (*Annona squamosa* L.) treated with star anise (*Illicium verum*) EO NE (IVNPs) at a chitosan:IVEO ratio of 1:1, with a MIC of 0.1 µL/mL against *A. flavus* and reductions in ergosterol levels of 93–100% [95]. Other studies further support that NE containing lemongrass EOs represent promising alternatives for maintaining the physicochemical and sensory quality of fruits during postharvest storage, such as avocado [97] and guava [103], potentially contributing to food waste reduction.

5.2. Meat Products, Fish, and Seafood

EOs incorporated into NE systems have been investigated in a wide range of meat products, including meatballs, lamb loin, chicken meat, and breaded products [104]. Different EO, including cardamom [92], cinnamon [91,99], cumin, safflower, clove, thyme, and rosemary [104], have been incorporated into NE aiming to control spoilage and pathogenic

microorganisms, including bacteria and fungi [92,104]. These systems have been applied both directly incorporated into meat product formulations and as edible coatings.

Bakr et al. (2024) [91] evaluated beef burgers treated with cinnamon EO NEs and observed severe morphological alterations in *A. flavus*, including mycelial disorganization, cellular collapse, and loss of plasma membrane integrity, as well as significant suppression of aflatoxin production. Sagar et al. (2022) [99] reported that chicken sticks coated with chitosan-based NEs containing cinnamon, clove, or thyme EO exhibited extended shelf life of up to 20 days (vs. 10 days for control), with minimum microbial counts of 4.7 log CFU/g. Lin et al. (2025) [82] developed a Salecan β-glucan-based edible film loaded with 5% lemon EO NE for chilled pork preservation. The film exhibited superior mechanical strength, barrier properties, and prolonged EO release, effectively controlling pH, total viable count, and lipid oxidation while extending shelf life [75]. According to Lotfy et al. (2023) [100], chitosan-based NE containing *Citrus limon* EO showed superior antimicrobial activity against *E. coli* in refrigerated ground meat compared to free EO and chitosan alone.

In fish and seafood products, the application of EO NEs remains relatively limited. EO derived from bay laurel [101], oregano, thyme, and lemongrass [90] have shown activity against microorganisms such as *Photobacterium damselae*, *Salmonella paratyphi* A, *Enterococcus faecalis*, *Vibrio vulnificus*, *Proteus mirabilis*, *Serratia liquefaciens*, and *Pseudomonas luteola* [101,105]. Yang et al. (2026) [106] investigated an oregano EO NE combined with ultrasound for multitarget control of *Vibrio parahaemolyticus* and its biofilm in clams. The NE alone promoted limited reductions (0.28 log CFU/g in tissue and 0.64 log CFU/cm^2 in biofilm), suggesting restricted system penetration. Mehraie et al. (2023) [107] evaluated chitosan coatings containing *Hyssopus officinalis* EO NE in seafood products, observing the lowest psychrophilic and mesophilic microbial counts after 12 days of storage (4.40 and 4.03 log CFU/g, respectively), significantly lower than the control. These effects were attributed to oxygen barrier function, disruption of the bacterial outer membrane, interaction with cell surface anionic groups, and antimicrobial activity of EO constituents (isopinocamphone, β-pinene, and pinocamphone).

5.3. Dairy Products

In dairy products such as milk, yogurt, cheeses, and fermented dairy beverages, NEs have been investigated as strategies for controlling filamentous fungi (*Aspergillus niger*, *Penicillium* spp., *Paecilomyces* spp.) as well as food-relevant bacteria, including *E. coli*, *S. aureus*, and *B. cereus* [59,88,108,109]. While some studies demonstrate effective and sustained microbial inhibition over time, comparable to conventional preservatives [108], others report limited or absent immediate antimicrobial activity, suggesting a delayed preservative effect associated with controlled release of active agents [110].

Although NEs containing natural compounds have gained attention for their antimicrobial potential in dairy matrices [88,108,111], their efficacy varies considerably depending on matrix complexity, target microorganism, storage conditions, and the release profile of bioactive compounds.

Kayiran et al. (2025) [110] evaluated *Thymbra spicata* EO (1–5%) in white-brined cheese and observed high antimicrobial activity in its free form, with inhibition zones ranging from 11.22 to 41.28 mm. In contrast, NEs containing the EO did not exhibit immediate antimicrobial activity, indicating that encapsulation promoted controlled release and resulted in delayed antimicrobial action compared with the free EO. In fermented dairy beverages, Shabani et al. (2025) [93] demonstrated that *Zataria multiflora* EO NEs (7.5% v/v) stabilized with Tween 80 exhibited bactericidal effects against *P. aeruginosa*, *E. coli*, *S. aureus*, and *B. cereus*, associated with morphological and cell wall alterations, maintaining microbial counts below detection limits during storage comparable to pasteurized control. In acidic

environments, the hydrophobic nature of EO compounds favors interaction with microbial membranes, leading to structural disorganization, leakage of intracellular constituents, and cell death [93]. Additionally, NEs exhibit improved antimicrobial efficiency due to reduced droplet size, increased surface area, and enhanced interaction with microbial cells, often mediated by hydrophilic groups of emulsifiers [93].

5.4. Beverages and Liquid Foods

In liquid products such as juices and functional beverages, the application of EO NEs has been widely investigated with emphasis on physicochemical behavior and system stability [112–114], aiming to prevent destabilization phenomena such as creaming, flocculation, and coalescence [42], as well as microbial proliferation [112–114]. Due to the relatively simple colloidal structure and high water content of these matrices, NEs enable more homogeneous dispersion of EO, minimizing phase separation during storage and transportation. Emulsifier selection represents a critical factor, particularly in acidic beverages, where proteins may undergo denaturation, aggregation, or loss of interfacial functionality [114].

Wang et al. (2016) [115] reported that a peppermint EO NE in a fermented dairy beverage exhibited high physicochemical stability and preserved product characteristics for 30 days at 4 °C. This stability was attributed to protein–polysaccharide complexes (electrostatic interactions, hydrogen bonding, and hydrophobic interactions), and the NE prevented microbial contamination without additional antimicrobial agents, highlighting its biopreservative potential. Zhang et al. (2025) [113] reported inhibitory effects of NEs containing rose EO against *E. coli* in cucumber juice. Similarly, Sun et al. (2025) [112] demonstrated the effectiveness of nanoemulsified *Litsea cubeba* EO in the inactivation of *Salmonella* Typhimurium in kiwi juice. Molet-Rodríguez et al. (2021) [114] demonstrated that, in an apple juice-based beverage, EO NEs exhibited reduced antimicrobial activity, characterized by slower inactivation kinetics and lower reductions in *E. coli*, which was associated with the protective effect of the sugar-rich matrix and limitations in the diffusion of bioactive compounds.

6. Safety, Toxicity, Migration, and Regulatory Considerations of Nanotechnology-Based Antimicrobial Systems in Food Matrices

6.1. Safety of Nanotechnology-Based Antimicrobial Systems in Food Matrices

Studies involving NEs and lipid-based nanocarriers have demonstrated that lipid-derived NEs formulated with food-grade emulsifiers generally exhibit low toxicity levels, showing substantially lower relative toxicity compared with inorganic or metallic nanomaterials. Lipid NEs tend to interact with metabolic pathways in a manner similar to conventional dietary lipids, which may reduce potential toxicological risks [116,117].

However, the safety of these systems cannot be generalized, as it depends on the structural characteristics of the food matrices into which they are incorporated. The physicochemical stability of NEs may be influenced by multiple factors, including water activity, the presence of proteins, pH variations, lipid content, salts, and molecular interactions within the system, potentially altering the release profile of bioactive compounds and impacting consumer exposure [42,118,119].

Furthermore, as highlighted by Priyadarshi et al. (2025) [120], safety assessment of nanotechnology-based antimicrobial systems must be multifaceted. It should not rely solely on the intrinsic toxicity of nanomaterials but also consider physicochemical transformations during shelf life, such as oxidation, ionic release, partial dissolution, and interactions with food matrix components. Although many nanomaterials comply with migration limits under laboratory conditions, significant knowledge gaps remain regarding chronic low-

level exposure and potential accumulation. Therefore, further systematic risk assessments are required, including migration studies, in vitro and in vivo toxicological evaluations, and advanced characterization techniques to ensure safe and sustainable implementation [120].

6.2. Toxicity and Bioavailability After Ingestion

The toxicity of nanotechnology-based antimicrobial systems is closely associated with their bioavailability following ingestion, primarily depending on transformations occurring during gastrointestinal metabolism. Upon ingestion, nanomaterials are exposed to a highly dynamic environment containing digestive enzymes, pH fluctuations, bile salts, and residual components derived from food matrices, all of which may induce significant alterations in their physicochemical properties, affecting aggregation, degradation, dissolution, and the ability to cross the intestinal barrier [121].

When considered individually, lipid-based NEs are generally more digestible and relatively less toxic than other nanomaterials, since their constituents exhibit structural similarities to conventional dietary lipids. These nanostructures undergo sequential enzymatic degradation mediated by lipases and bile salts, ultimately being released as fatty acids and monoglycerides. However, NEs tend to significantly enhance the bioavailability of lipophilic compounds, with increases of approximately two- to four-fold reported in advanced digestion models, while lipophilic flavonoids may exhibit increases of up to 14-fold and curcumin up to nearly 70-fold [122–124]. Although such improvements enhance biological activity, they may also potentiate undesirable effects when consumed at high doses or over prolonged exposure periods, reinforcing the importance of precise control over formulation design and applied dosage [125].

In light of these findings, the importance of conducting specific toxicological assessments tailored to different nanomaterials and their respective applications can be strongly emphasized [126,127].

When considering interactions between nanomaterials and food matrices, additional complexities arise in toxicity assessment. These interactions may act as inhibitors or enhancers of toxicity by altering bioavailability, depending on nanoparticle type, lipid content, protein composition, and aggregation behavior. Proteins, polysaccharides, and dietary fibers have been shown to reduce the bioaccessible fraction of nanomaterials, whereas lipids may enhance absorption [117,119]. Thus, the food matrix is a critical determinant of the toxicological behavior of these systems.

6.3. Regulatory Considerations and Market Acceptance

Significant divergences exist among countries and economic blocs regarding regulatory frameworks applied to nanotechnology-based antimicrobial systems, largely due to differences in risk assessment methodologies. In the European Union, risk assessments are conducted on a case-by-case basis by the European Food Safety Authority (EFSA), requiring comprehensive physicochemical characterization of nanomaterials, including detailed migration studies, toxicological data, and system behavior throughout the product shelf life [128,129].

NEs formulated with common food ingredients (oils, surfactants, and biopolymers) generally face lower regulatory barriers and are often considered generally recognized as safe (GRAS), provided that no new properties arise that substantially alter consumer exposure profiles. Nevertheless, regulatory authorities require specific evidence demonstrating potential effects associated with structural size reduction, unexpected increases in bioavailability, and possible new risks to human health [130,131].

Successful regulatory implementation of nanotechnology-based antimicrobial systems depends on the integration of food science and technology, toxicology, materials engineer-

ing, and public policy. Rigorous and reproducible data, balanced regulatory policies, and appropriate risk assessments tailored to each class of nanomaterials and food matrices are essential for consumer protection and for fostering technological advancement in the food sector [132,133].

7. Challenges and Knowledge Gaps

One of the main gaps identified in the literature lies in the fact that most studies evaluating the antimicrobial activity of NEs are conducted in simplified model systems, without considering the inherent complexity of real food matrices. Although such investigations are essential for understanding the physicochemical and biological behavior of NEs, knowledge regarding their effectiveness when applied to real food products remains limited. Food systems are intrinsically heterogeneous and may significantly affect NE stability as well as the accessibility of antimicrobial compounds to target microorganisms.

Fermented products, acidic foods (such as industrial sauces, including tomato sauce and ketchup), low-water activity matrices (snacks, cereals, and bakery products), high-fat food systems, as well as dairy and plant-based beverages, remain poorly explored compared to studies conducted in culture media or simplified food systems. Furthermore, studies investigating the interactions among multiple microbial species naturally present in foods are still scarce, as are investigations addressing the influence of food matrix composition on NE physicochemical stability and antimicrobial compound release kinetics. Additional gaps include the impact of storage conditions, particularly temperature and shelf-life duration, on the maintenance of antimicrobial activity, as well as the structural stability of NE following freezing–thawing cycles in frozen products. Understanding these factors in future research is essential to accurately predict the technological and antimicrobial performance of NE under real application conditions.

Another aspect that deserves attention is the lack of technological strategies aimed at mitigating potential negative sensory impacts associated with the incorporation of NEs containing EOs. Studies attempting chemical modifications of these compounds to preserve their antimicrobial properties may inadvertently alter sensory perception, particularly in food matrices with delicate sensory profiles, such as certain fresh cheeses. In such systems, the incorporation of NEs may modify the characteristic flavor and influence attributes such as texture and color, ultimately compromising overall product acceptance [115,118]. In this context, studies focused on structural adjustment of bioactive compounds to maintain antimicrobial efficacy without intensifying aromatic perception remain scarce in the scientific literature.

The absence of standardized protocols for EO extraction and concentration, combined with the intrinsic variability of plant materials, affected by geographic origin and plant developmental stage, hinders direct comparison among studies employing the same type of EO. Even when derived from the same botanical species, EOs may exhibit significant variations in chemical composition and concentrations of active compounds, directly influencing the observed antimicrobial activity. Experimental variability among studies is also evident, including differences in microbial strain selection, inoculum concentration and physiological state, exposure time, storage conditions, food matrices, analytical methods, and physicochemical characterization parameters. Such variability compromises quantitative comparisons and evidence-based meta-analyses [40,100,106,110,134,135]. The identification of bioactive compounds responsible for antimicrobial activity, followed by their isolation and evaluation in purified form, could contribute to improved reproducibility and experimental standardization. From a scientific perspective, this approach would facilitate comparison among studies and allow the establishment of more accurate dose–response relationships.

Emerging Evidence on Adaptive Responses and Antimicrobial Resistance

While the multi-target mechanisms of EOs are often cited as limiting classical resistance development [13,14], recent studies suggest that sublethal exposure may still induce adaptive responses or tolerance in foodborne pathogens. For instance, *Y. enterocolitica* exposed to sublethal concentrations of oregano EO upregulated genes involved in antioxidant defenses (glutathione peroxidase) and membrane integrity, while repressing energy-demanding processes such as motility and quorum sensing [23]. Similarly, *L. monocytogenes* exposed to thymol and cinnamaldehyde activated alternative energy-generating pathways and repressed virulence genes [31]. These transcriptomic reprogramming events, while not constituting genetic resistance, may enhance bacterial survival under sublethal stress conditions encountered during food processing or storage. Complementing these findings, *Salmonella* Typhimurium exposed to sublethal concentrations of thyme and cinnamon EO developed direct- and cross-resistance (to thermal and oxidative stress), with 161–324 differentially expressed genes, leading the authors to recommend caution in the use of EOs in the food industry, as sublethal stress may promote resistance development [136].

Of greater concern is the potential for cross-resistance to clinically relevant antibiotics. Recent evidence indicates that EO can modulate efflux pump activity in multidrug-resistant pathogens. Studies have demonstrated that EO components can synergize with antibiotics by affecting efflux pump expression in foodborne pathogens and clinical isolates, with garlic, cinnamon, and thyme EOs significantly reducing efflux pump activity and enhancing antibiotic sensitivity [137,138].

NE-based delivery may further complicate this landscape. Research has shown that EO NEs can significantly reduce biofilm formation by multidrug-resistant clinical strains, with nanoencapsulation enhancing penetration into the biofilm matrix and potentially overcoming one mechanism of antimicrobial tolerance [139]. However, the long-term effects of chronic sublethal exposure to nanoemulsified EO on microbial populations remain unknown.

Several critical knowledge gaps persist regarding EO NEs and antimicrobial resistance:

(i) The effects of chronic sublethal exposure to nanoemulsified EOs on microbial population dynamics during food storage;
(ii) Whether repeated use in food systems could select for tolerant strains or induce stable genetic changes;
(iii) The potential for horizontal gene transfer of resistance determinants following EO exposure;
(iv) The impact of EO NEs on non-target microbiota, including beneficial microorganisms;
(v) The minimum selective concentration (MSC) at which EO NEs may promote adaptive responses.

Addressing these gaps requires long-term exposure studies, genomic and transcriptomic analyses (including whole-genome sequencing of adapted strains), and systematic risk assessments under realistic food processing and storage conditions [140]. Future research should prioritize understanding the concentration thresholds below which EO NE remain effective without inducing tolerance, as well as the potential synergy or antagonism between nanoemulsified EO and conventional preservatives or antibiotics.

Consistent with these gaps, most available studies remain focused on evaluating antimicrobial activity against specific pathogens, such as *Salmonella* spp., *E. coli*, *S. aureus*, and *L. monocytogenes* [21,58,92,105], as well as certain bacteria relevant to seafood products [101,105] and fungi of technological importance, such as *A. flavus* [91].

In contrast, studies addressing natural microbial communities present in foods remain extremely limited. Existing investigations are largely restricted to microbial enumeration, such as mesophilic bacteria, lactic acid bacteria, and molds/yeasts, in cheeses treated

with NE, without deeper analysis of microbiota composition, population dynamics, or effects on beneficial microorganisms [110]. Consequently, the indiscriminate application of nanostructured antimicrobial agents may promote microbial tolerance and accelerate the development of resistance in both target and non-target microorganisms, reinforcing the need for systematic studies assessing risks, usage limits, and ecological impacts associated with these nanomaterials [140].

8. Final Considerations

Essential oil nanoemulsions (NEs) offer significant technological advantages for controlling foodborne pathogens and spoilage microorganisms, including improved solubility, enhanced stability, and increased antimicrobial efficacy compared to free essential oils. However, a major challenge remains: the discrepancy between their performance in simplified model systems and their actual efficacy in complex food matrices, where components such as proteins, lipids, and polysaccharides can modulate NE stability and bioactivity.

This review examined the importance of food matrix effects, the mechanisms of antimicrobial action, and the safety and regulatory aspects of NE. Key findings indicate that while NE often outperforms free essential oils, their efficacy is highly dependent on matrix composition, pH, ionic strength, and processing conditions. Safety evidence remains limited, particularly regarding chronic exposure and long-term effects.

Future research should prioritize: (i) standardization of protocols for NE preparation and testing; (ii) studies in multi-component food systems that better represent real-world conditions; (iii) assessment of industrial application challenges, including scalability, cost, and regulatory compliance; and (iv) investigation of adaptive responses and antimicrobial resistance risks following sublethal exposure, as well as ecological impacts on non-target microorganisms.

In conclusion, the successful translation of NE into industrial biopreservation tools will depend on integrating efficacy, stability, safety, sensory acceptability, and regulatory compliance under practical conditions. Particular attention should be given to understanding the potential for adaptive responses and antimicrobial resistance following sublethal exposure, as well as the long-term ecological impacts on both target and non-target microorganisms.

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