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Recent trends and advancements in nanoemulsions: Production methods, functional properties, applications in food sector, safety and toxicological effects

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ABSTRACT

Nanoemulsions - colloidal dispersions of nanoscale droplets in a continuous phase, have garnered significant attention in recent years due to their versatile properties and wide-ranging applications. This review paper presents a comprehensive overview of nanoemulsions production methods focusing on their formulation strategies, functional properties and different applications in food processing. Various low and high energy methods such as phase inversion, high pressure homogenization, ultrasonication and microfluidization etc., are reviewed to produce nanoemulsions with diverse characteristics and functionalities. Scale-up potential and practical challenges of each production method are also discussed. Various properties of nanoemulsions stability, drug solubility, bioavailability and targeted delivery are highlighted. Furthermore, their potential applications in food and beverage sectors including encapsulation of bioactive compounds, packaging, preservation and new product formulation have been elaborated along with real world examples at commercial stage. This review will contribute to providing knowledge about gastrointestinal fate of nanoemulsions, toxicity and safety by in-vitro and in-vivo testing as well as other research evidences. Regulatory aspects and public perception on use of nanoemulsions are also covered. Nevertheless, nanoemulsion presents immense potential for addressing various challenges in food industry. Continued research efforts in formulation design, ingredient selection, its compatibility with food constituents as well as the associated safety issues should be undertaken to unlock new opportunities towards scalability and commercialization.

1. Introduction

Nanoemulsions are isotropic, kinetically stable, thermodynamically unstable, transparent colloidal dispersions consisting of oil and aqueous phase, surfactant and a cosurfactant with droplet size within the range 20–200 nm [56]. They are much better kinetically stable to gravitational separation and aggregation than other emulsions. The thermodynamic instability is because of the large positive interfacial tension between the oil and water phases. They appear transparent since very little scattering of the visible light

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takes place due to the smaller droplet size of nanoemulsions as compared to the wavelength of visible light. All these characteristics are dependent on conditions, such as, composition, pressure, temperature, and the preparation techniques. Also, the nature of the dispersed phase, dispersion medium, surfactant and cosurfactant have substantial effect on the formation of stable nanoemulsions. Therefore, there is great possibility to prepare nanoemulsions with tuneable properties and required droplet sizes focussing on varied applications. Nanoemulsions have been gaining considerable demand for fundamental researches and applications in numerous fields including food, cosmetics, health care, pharmaceuticals, agrochemicals, and biotechnology [34,13].

Nanoemulsions are being considered as a potential candidate in the field of food processing and packaging. They impart excellent properties such as high oil in water interfacial tensions, high nutrient bioavailability, digestibility, stability, non-toxicity and non-irritant behavior [78]. They are widely applied for encapsulation, protection and delivery of bioactive lipophilic components, such as vitamins, antioxidants, antimicrobials, drugs, nutraceuticals and other functional compounds within our body. Encapsulation of nanoemulsions includes incorporation, absorption, or dispersion of bioactive compounds within small capsules having diameters less than 100 nm. They are also useful in the controlled release of coloring and flavoring compounds in foods. Nanoemulsion based edible nanocoatings containing various bioactive agents can be used to coat foods such as fresh produce, fresh cut fruits and vegetables, meats, dairy products such as cheese and confectionaries to improve their shelf life. The nanoemulsion coatings can also prevent moisture and gas exchange, minimize oxidation and browning action in foods.

The bioactive compounds in nanoemulsion should have targeted delivery and need to be bioavailable to exert their effects in-vivo. It is thus important to study the gastrointestinal fate of nanoemulsion at different platforms of the nutrient digestion. Besides, the use of nanomaterials could present some potential risks. The nanoemulsions utilized in food interact with the human body, through oral ingestion, skin contact, or injection routes. Considerable efforts are being directed toward understanding the potential toxicity of ingested nanoparticles.

This review emphasizes on different routes of nanoemulsion synthesis, properties and challenges associated with production. Also, it primarily focusses on technological advancement and research in domain of nanoemulsions' applications in food industries. Recent trends on commercial use have also been presented in order to offer practical insights and scale-up potential. The gastrointestinal fate of nanoemulsions through in-vitro and in-vivo testing is also highlighted. The safety concerns, public perceptions and regulatory aspects relating nanoemulsion are also discussed.

2. Production of nanoemulsions, scalability issues and practical challenges

Selecting an appropriate technique for nanoemulsion synthesis depends on specific compound properties, such as the homogenization of oil phases and surfactants, operational characteristics, and physicochemical properties of the product [88]. A critical aspect of nanoemulsion production involves attainment of extremely low interfacial tension (< 10–3 mN/m) at the oil-in-water (O/W) interface, necessitating the appropriate utilization of surfactants. These surfactants play a pivotal role in stabilizing droplets with low interfacial tension. Additionally, fluidity significantly contributes to promoting nanoemulsion formation at the interface. The preparation of nanoemulsions can be categorized into two main approaches; low energy and high energy emulsification methods depending on how energy is delivered to the system being emulsified [88,119,122].

2.1. Low-energy methods

They utilize the internal physical properties of the system to form nano droplets [47]. The emulsification process in these methods can be induced by altering parameters such as temperature and composition, thereby influencing the hydrophilic-lipophilic balance of the system [121,21]. Two main methods are commonly reported for the development of nanoemulsions using the low-energy approach, namely spontaneous emulsification and phase inversion [88,31].

2.1.1. Spontaneous emulsion (SE)

Spontaneous emulsion also known as solvent diffusion emulsification (SDE), operates through various mechanisms [38,71]. Primarily, it is a diffusion-driven process occurring when two immiscible liquids are mixed under non-equilibrium conditions due to a chemical potential gradient among the phases [143]. This method can be controlled by either changing the temperature without varying the composition or keeping the temperature constant while altering interfacial properties and compositions. Notably, they can be formed under ambient conditions without use of any specialized equipment. However, limitation of this approach is the presence of the solvent and small quantity of the oil phase [88]. Although the industrial utilization of this procedure is still in its infancy, it has demonstrated potential as a cost-effective method, producing small droplets measuring 10 nm [23].

2.1.2. Phase inversion

Phase inversion temperature, phase inversion composition, and emulsion phase inversion (EPI) technologies are widely recognized as popular phase inversion techniques in the food industry [95]. Phase Inversion Temperature (PIT) is the temperature at which the transition from oil-in-water (O/W) to water-in-oil (W/O) emulsion occurs, utilizing the molecule invasion property where emulsifiers change their hydrophilicity or lipophilicity as a function of temperature at a constant composition [14] (Fig. 1A). This technique enables the production of nanoemulsions with low-energy input without the use of high shear forces. Emulsion phase inversion (EPI) refers to the transformation of O/W emulsions to W/O emulsions or vice versa, representing a desirable and dynamic phenomenon based on phase transition during the emulsification process [54]. These phase transitions are instigated by the surfactant's impulsive curvature and can be persuaded by modifying the properties of non-ionic surfactants through temperature changes

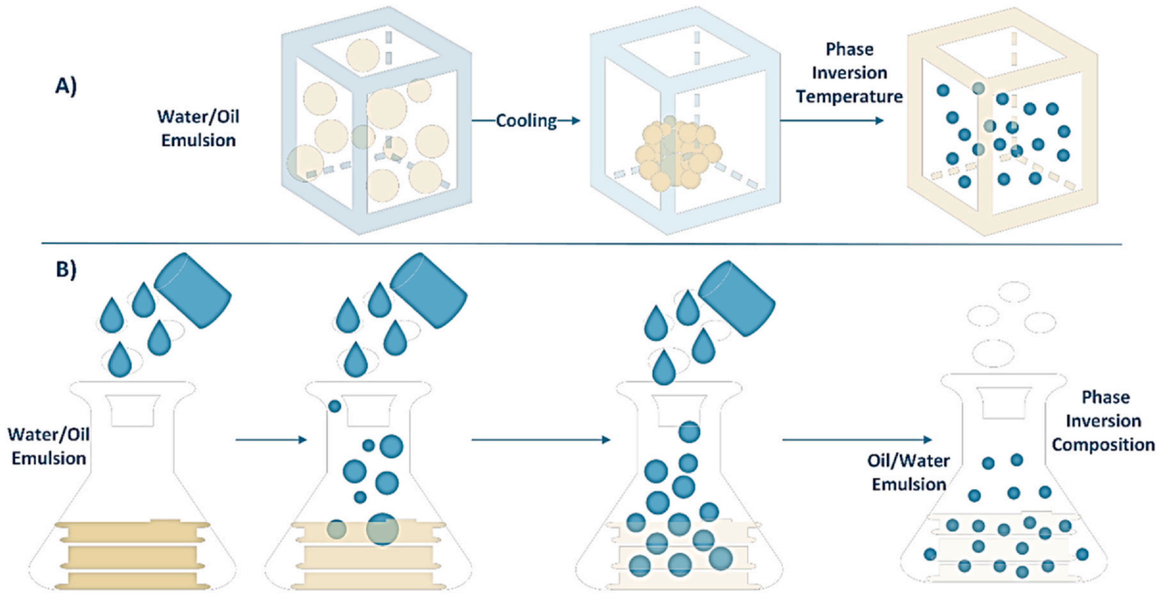

Fig. 1. Overview of nanoemulsion formation through a low energy approach, (A) Phase Inversion Temperature; (B) Phase Inversion Composition.

or alterations in system composition. The phase inversion method is influenced by several parameters such as salt concentration, oil fraction or water, temperature, energy input, or variations in formulation parameters (temperature, salinity, etc.) [88]. Phase Inversion Composition (PIC), also known as emulsion inversion point, involves altering the emulsifier's hydrophilic-lipophilic behaviour by varying the ingredient makeup (Fig. 1(B)). When salt is introduced to O/W nanoemulsions containing an ionic emulsifier, the electric charge of the surfactants is modified, resulting in a W/O emulsion system. Similarly, W/O emulsions can be converted to O/W by diluting with water. However, this method is challenging to apply to hydrophobic compounds [47,88,13]. Another approach in preparing nanoemulsions through PIC involves modifying the electrical charge (pH modification) of the emulsion [78]. Pagan et al. [101] utilized the PIC method to prepare nanoemulsions incorporating citral, evaluating their antimicrobial activity. The study demonstrated that the citral nanoemulsion exhibited greater effectiveness than free-form citral [31]. In comparision to PIC method, PIT method generally exhibits a low polydispersity index and high emulsification efficiency [58].

2.2. High-energy procedures

In high-energy emulsification methods, nanoemulsions can be prepared using mechanical devices, such as, high-pressure homogenizer, microfluidizer or ultrasound generator [47] (Fig. 2). These devices are capable of generating strong disruptive forces to increase the water/oil interfacial area for the formation of small droplets [119]. The droplet size obtained through these high-energy methods is dependent on the intensity of energy applied. As the high-energy approach dissipates a substantial amount of energy through friction losses caused by high shear rates, this energy is transformed into heat, leading to an increase in the emulsion temperature [88,157]. The processes involved in high energy procedure are described below.

2.2.1. Ultrasonication

The ultrasonication technique depends on soundwaves of high frequency of 20 kHz and above. An oil and aqueous phase coarse emulsion is subjected to cavitation using the ultrasonication method using high-frequency sound waves. As the acoustic waves travel through the emulsion, they produce cycles of high pressure (compression) and low pressure (rarefaction). Small vapor-filled bubbles form and enlarge during the rarefaction phase and during the compression phase these bubbles collapse resulting in release of considerable amounts of energy further into shock waves and microjets. This mechanism, known as cavitation, creates a stable nanoemulsion by efficiently dissolving larger oil droplets into much tiny droplets, usually less than 100 nm. Multiple parameters, such as sonication strength, time, and emulsifier content, influence the size and dispersion of the resultant droplets. The application of ultrasonication process for nanoemulsion preparation has garnered interest due to its energy efficiency, the ability to use low-end mixing instruments, the ease of manipulating the system, and, notably, its low production cost [82]. This technique also uses less energy compared to other high-energy techniques [88]. This technique is notable for its lower energy requirements and the ability to handle sensitive bioactive compounds at reduced temperatures, thereby preventing thermal degradation. The produced ultrasonic waves (Fig. 2 (A)) efficiently disperse the oil phase into the water phase by the mechanism of cavitation, resulting in the formation of monodisperse droplets with diameters less than 100 nm [135]. The process reduces the size of droplets by adjusting sonication duration, power level, emulsifier concentration, and the type and content of the oil phase. Although the process has great potential, the preparation method is limited to small batches, thus remains restricted to lab scale experiments [21].

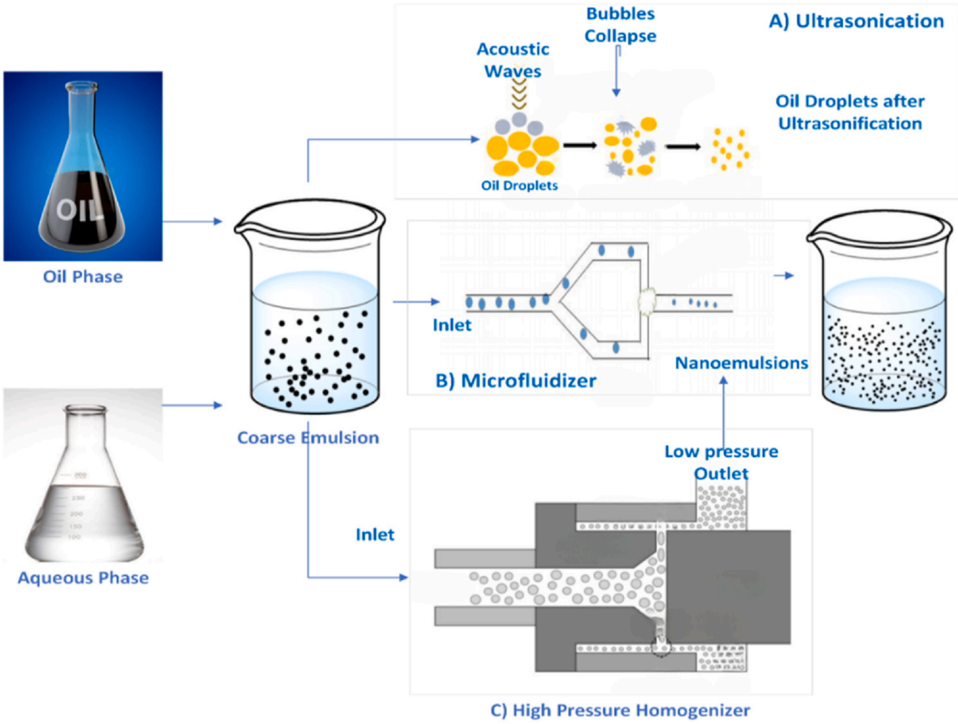

Fig. 2. Schematic diagram of nanoemulsion formation using high-energy approach.

Nonetheless, the application of ultrasonication process for nanoemulsion preparation has garnered interest due to its energy efficiency, the ability to use low-end mixing instruments, the ease of manipulating the system, and, notably, its low production cost [82]. This technique also uses less energy compared to other high-energy techniques [88]. Focused-ultrasonic acoustics can prove to be a highly efficient technology for assisting in the formulation of nanoemulsions [84]. Dispersions generated by ultrasound exhibit enhanced stability, and the resulting small droplets display an excellent particle size distribution [140]. The use of ultrasound in nanoemulsion formation has shown promising results, as it eliminates the need for additional requirements in the emulsion manufacturing process [91]. Shao et al. [133] employed ultrasonication to prepare a new eugenol nanoemulsion. The resulting droplets exhibited a regular spherical shape with size ranging from 80 to 100 nm. Moreover, the nanoemulsions demonstrated high storage and thermal stabilities, along with excellent antioxidant capacity and antimicrobial activity [31].

2.2.2. Microfluidization

Microfluidization is a distinctive mixing technique that concurrently diminishes particle size through processes such as attrition, impact, hydraulic shear, impingement, severe cavitation, and turbulence [122]. The method of creating nanoemulsions by the use of a specialised microfluidizer device is known as microfluidization, which is a high-shear technique that is sophisticated. In this method, while the emulsion moves through microchannels in the microfluidizer, it is subjected to very high velocities that result in large shear forces and pressure gradients. This microfluidizer device incorporates a high-pressure displacement pump (operating in the range of 500 – 20,000 psi) through which materials are directed into a chamber containing multiple microchannels with dimensions ranging from 50 to 300 µm (Fig. 2 (B)). As the materials traverse the microchannels and reach an impingement area, they undergo a transformation into very fine particles. The manufacturing process utilizing microfluidization typically begins with the liquid phases, consisting of oil and aqueous phases, being processed in an inline homogenizer to yield a coarse emulsion. Subsequently, this coarse emulsion is passed through the microfluidizer, leading to the production of a fine nanoemulsion. This cycle is repeated as necessary until the desired particle size is achieved. While microfluidizers allow for the formation of approximately monodisperse droplets, they have some disadvantages, including high manufacturing costs compared to other high-energy equipment. This technique can result in enlarged droplet sizes due to coalescence caused by the extended time taken in the emulsification process and an increase in temperature due to the use of high pressure in nanoemulsion production. This method's ability to offer a regulated and reliable emulsification environment makes it especially useful for producing stable nanoemulsions with limited size distributions. However, compared to other high energy methods, the disruption of droplets by the microfluidization procedure is higher, resulting in the formation of fine droplets with a uniform size [88,14]. Microfluidization excels in its ability to precisely control droplet size through adjustable shear rates and pressure gradients, enabling consistent and reproducible production, particularly valuable for large-scale industrial applications. This method is known for producing uniform nanoemulsions with minimal variability between batches. Microfluidizers prove to be a valuable tool in the preparation of nanoemulsions containing active ingredients for the production of edible films, imparting various physical and functional properties [2,5].

2.2.3. High pressure homogenization

High pressure homogenization (HPH) is a popular method for preparation of nanoemulsions due to its scalability and flexibility. The technique relies on the hydraulic shear and cavitation phenomenon to disrupt and produce smaller-sized oil droplets from a previously prepared coarse emulsion. In the process, the emulsion including surfactants and cosurfactants are passed through a small orifice of piston homogenizer under high pressure (500–5000 psi) to produce nanoemulsions (Fig. 2 (C)). The mean droplet size and particle distributions can be significantly affected by factors such as homogenization pressure and the number of cycles [127]. HPH is useful for decreasing droplet size and the polydispersity of oil droplets [21]. It is a simple and scalable process extensively used to produce fine nanoemulsions and of high stability for a long time Its ease of application, scalability, reproducibility, and high throughput with better quality make it highly suitable for producing nanoemulsions in the food industry [70,14].

2.2.4. Rotor-stator emulsification

The rotor-stator emulsification (RSE) is the technique used in the food industry to produce coarse emulsion prior to further disintegration steps carried out in homogenization or microfluidization. The driving force for this high-speed stirring technique is hydrodynamic shear. The size of the droplet depends on the rotor design, rotor speed, gap size and time of treatment [69,21]. It has several advantages, such as, rotor stator devices are relatively easy to install into existing vessels and tanks and require comparably low costs of investment. This type of process is often desired in large scale production because of its higher process efficiency: higher product output can be achieved in a shorter production time. These devices also offer compatibility with viscous systems, and the ability to prepare large volumes of emulsions [88].

2.3. Limitations and practical challenges of production methods

The major practical limitation of microfluidization is thermal degradation of heat-sensitive ingredients due to the intense shear forces and pressure gradients ultimately limit the applicability of this method for certain formulations [14]. The high energy requirement leads to significant operational costs, especially for large-scale production. Additionally, the requirement for multiple passes to achieve uniform droplet sizes can further increase processing time and energy consumption. The equipment itself is also relatively expensive and complex, requiring skilled operation and maintenance. Also, the potential for clogging in the microchannels necessitates careful formulation and pre-processing of the emulsion to ensure smooth operation, adding another layer of complexity to the process. The primary limitations of ultrasonication for nanoemulsion production include its scalability constraints due to low capacity of the equipment and high energy input required. Additionally, there is a risk of metal contamination from the ultrasonic probe and potential degradation of sensitive components, such as proteins and emulsifiers, due to prolonged exposure to ultrasonic waves [88]. The high energy costs further limit its feasibility for large-scale production. The demerit of HPH technique is that it is suitable for preparing O/W nanoemulsions containing less than 20 % oil phase. However, this technique becomes unsuitable when formulating high-viscosity or creamy nanoemulsions with mean droplet diameters below 200 nm [134]. RSE is probably the least favorable method for the one-step production of nanoemulsions. It is very difficult to achieve droplet sizes below 1 μ with this method. Also, the agitated vessels and high-shear mixers offer inhomogeneous power input, which results in wide emulsion droplet size distributions [150]. Nevertheless, many of the nanoemulsions components regardless of whether prepared through low-energy or high-energy methods, are not suitable for application in the food sector. This includes synthetic components like surfactants, polymers, synthetic oils, or organic solvents. Therefore, it is essential to use food-grade ingredients which are legally accepted, label-friendly, and economically viable for e.g flavor oils, triglyceride oils, proteins, and polysaccharides in the food nanoemulsions formulation. Synthetic surfactants employed as emulsifiers in the formulation of nanoemulsions may introduce off-flavors or contribute to the overall toxicity of the end product [29].

3. Functional properties of nanoemulsions

3.1. Solubilization capacity

Nanoemulsions indeed hold great promise for revolutionizing the oral drug delivery system, addressing several limitations associated with traditional systems. This innovative approach has provided a comprehensive solution to challenges related to drug solubility, absorption rates, and targeted drug delivery. Nanoemulsion formulation has been employed to enhance the solubilization of phytosterols. Additionally, nanoemulsion formulations have been demonstrated to increase the solubilization of lycopene [43,119]. In terms of drug solubility, both hydrophilic and lipophilic drugs can be effectively solubilized in nanoemulsions, whether they are of the O/W or W/O type. This versatility ensures enhanced dissolution due to the extremely small size of the particles, which possess both hydrophilic and lipophilic components. The reduced particle size facilitates improved drug solubility and bioavailability [145].

3.2. Bioavailability

The suboptimal bioavailability of certain naturally occurring active compounds poses a hindrance to their effective pharmacological activities. Nanoemulsions have proven to be a viable solution, serving as an appropriate form to enhance the bioavailability of natural extracts. Curcumin nanoemulsions demonstrated noteworthy inhibition of 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation. Nevertheless, the oral administration of curcumin faces challenges due to its low bioavailability. To address this issue, flavored nanoemulsions have been formulated, showcasing enhanced curcumin digestibility when compared to the direct consumption of curcumin [119]. Moreover, the nanoemulsion formulation of oil-soluble vitamins, such as alpha-tocopherol, has been shown to augment their oral bioavailability and enhance pharmacological effects [119]. Furthermore, the bioavailability of quercetin or methylquercetin has been enhanced through nanoemulsion preparations. Sari et al. [124] conducted a study incorporating curcumin into the oil droplets of medium-chain triglycerides using whey protein concentrate-70 and Tween-80 as emulsifiers. The in-vitro release kinetics indicated the relative resistance of the nanoemulsion against digestion with pepsin. However, when subjected to pancreatin, the compounds were released. The authors concluded that nanoencapsulation of highly unstable lipophilic compounds is an effective approach to enhance certain properties, such as bioavailability. Silva et al. [138] conducted a study where they incorporated curcumin into a multilayer nanoemulsion using layers of alginate and chitosan. The simulated in-vitro digestion conditions indicated that this type of nanoemulsion provided improved control over the speed and degree of digestibility compared to uncoated nanoemulsions [31]. In a study, pectin-based nanoparticles prepared from nanoemulsion templates were employed to improve dissolution and absorption property of itraconazole (ITZ), a poor water-soluble drug. These templates were prepared through a high-pressure homogenization method, utilizing pectin as an emulsifier and chloroform as the oil phase. The in-vivo absorption study, conducted in fasted rats, revealed improved absorption of ITZ with nanoparticle prepared from nanoemulsion templates and it was 1.3 times higher than that observed with the commercial ITZ product [20,145].

3.3. Stability

3.3.1. Physical stability

The nanoemulsions experience thermodynamic instability because of the hydrophobic effect which causes undesired molecular interactions at the oil-water interface. Nanoemulsions may degrade through various mechanisms, including flocculation, gravitational separation, coalescence, phase separation, and Ostwald ripening [47,53,80,31]. In gravitational separation, due to density difference nanoemulsion droplets separate out from the surrounding liquid causing sedimentation or creaming. In flocculation, two or more droplets attract each other to form clusters, while in coalescence, larger droplets are formed after droplets collision and amalgamation. In Ostwald ripening, the larger droplets grow at the expenses of smaller ones. Arancibia et al. [10] ascribed the diminished stability of nanoemulsions prepared with avocado oil (15 %) and starch (8 %) to gravitational separation resulting from the fat droplet flocculation/coalescence and precipitation/aggregation of starch. Similarly, Chen et al. [22] noted incapability of pure cinnamaldehyde to produce stable nanoemulsions due to higher density (1050 kg.m^{-3}) than room temperature water (997 kg.m^{-3}). These differences cause gravitational separation (sedimentation) which can be avoided by mixture of cinnamaldehyde and triglycerides. Bai et al. [18] reported emulsion instability with use of amphiphilic polysaccharides above a certain level as emulsifiers due to exhausted flocculation mechanism. Li & Lu [68] observed that D-limonene nanoemulsions tended to become unstable, exhibiting flocculation and coalescence, leading to variations in zeta-potential at different storage temperatures. Bai et al. [18] found that rhamnolipids could stabilize O/W nanoemulsions, maintaining stability under various conditions. However, under extremely acidic conditions (pH 2–4) or in the presence of high ionic strength (200–500 mM NaCl), coalescence was identified as the primary destabilization mechanism due to weakening in electrostatic repulsion among the droplets [71].

3.3.2. Chemical stability

Nanoemulsion may undergo various biochemical and chemical reactions, such as lipid oxidation, flavour loss, biopolymer hydrolysis and discoloration, which can result in the loss of their desirable properties. Lipid oxidation is regarded as a highly significant type of chemical breakdown among them [156]. The relatively large interfacial areas of nanoemulsions can accelerate this lipid oxidation as water-soluble pro-oxidants (e.g., transition metals) come into contact with oil-soluble reactants (e.g., polyunsaturated lipids and hydroperoxides). Park et al. [103] suggested that the long-term stability of nanoemulsions is mostly influenced by chemical stability rather than physical stability, particularly in the context of O/W nanoemulsions containing retinol. Three commonly employed methods to improve the chemical stability of nanoemulsions are: Manipulation of interfacial characteristics, incorporating substances with chelating or antioxidant properties can help mitigate chemical degradation, especially lipid oxidation; Control of environmental elements: managing factors such as temperature, light exposure, pH, and oxygen levels can contribute to maintaining the stability of nanoemulsions during production, storage, transportation, and application [70].

3.3.3. Correlative instability mechanism

The stabilization mechanism of nanoemulsions is closely related to their physicochemical properties, including composition, interfacial composition, electric charge, droplet size, physical state, aggregation state, rheology properties, and more [53,164]. Multiple instability mechanisms are related and can appear simultaneously. For example, Powell et al. [106] observed destabilization in nanoemulsions, which were prepared using Pluronic F68 and different types of oil, as a consequence of the droplets growth caused by Ostwald ripening and coalescence. The Ostwald ripening prevails during the first phase when droplet sizes were small and is then followed by droplet growth through coalescence for a significant longer time. To prevent droplet aggregation caused by flocculation and coalescence, it is essential to ensure that the repellent interactions between droplets such as electrostatic and steric forces are stronger than the attractive interactions like van der Waals, hydrophobic, and depletion forces. This can be achieved by adjusting the composition of the aqueous phase or the nature of the emulsifier used. Commonly used emulsifiers include low molecular weight surfactants (mono and diglycerides, derivatives of monoglycerides, polyoxyethylene derivatives such as Tween series and span series, phospholipids, glycolipids, saponins etc.) and high molecular weight emulsifier comprising of protein, polysaccharides and their mixtures or conjugates etc. Additionally, the use of ripening inhibitors, wetting agent, texture modifiers or selecting an oil phase with low water-solubility can help limiting Ostwald ripening, contributing to the overall stability of nanoemulsions [163]. [159] utilized ultrahigh pressure homogenization to develop nanoemulsions, employing soy protein isolate (SPI), β-conglycinin (7S), or glycinin (11S) as emulsifiers. These nanoemulsions demonstrated excellent stability under various conditions, including different ionic strengths (0–500 mM NaCl), temperatures (30–60°C), pH levels (< 4 or > 7), and storage durations (0–45 days). Besides, the type of emulsifier also had a significant impact on the stability of nanoemulsions. Surfactants contribute to stability through different mechanisms, with ionic surfactants providing electrical charge and non-ionic surfactants creating a steric barrier with bulky molecular groups [137]. The stability of emulsions against Ostwald ripening has been demonstrated to increase significantly with a decrease in the hydrocarbon solubility in water as the dispersion medium [128,25]. Delmas et al. [32] demonstrated reduction in Ostwald ripening in oil blend of mono-, di-, and triglycerides even at high temperature by adding wax to it. However, in this trapped species method, the presence of immobile species in the continuous phase restricts the size reduction. This drawback can be overcome by another method called evaporational ripening in which O/W emulsion can be prepared using an oil phase that consists of a polymer dispersed in a highly volatile solvent [142]. Nam et al. [89] demonstrated that O/W nanoemulsions can be successfully stabilized against Ostwald ripening, by using surfactants that form a physically robust interfase for e.g. ampiphilic block copolymer polyethylene oxide-polycaprolactone. In addition to regular stability and accelerated stability studies recommended by ICH (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use), nanoemulsions undergo specific thermodynamic stability assessments to ensure droplet integrity in response to temperature fluctuations [142].

3.4. Controlled release

Nanoemulsions, with their small-sized nanoparticles, offer the advantage of easy passage through pores. Additionally, the presence of both hydrophobic and hydrophilic units facilitates penetration through both the hydrophobic stratum corneum and hydrophilic sweat ducts [145]. In the case of ropinirole, a drug characterized by low oral bioavailability and requiring frequent dosing, the preparation of a nanoemulsion gel has shown improved penetration and extended release. A study demonstrated that the relative bioavailability of ropinirole was increased by more than two-fold compared to a conventional marketed gel, highlighting the potential of nanoemulsion gel formulations in enhancing drug delivery and bioavailability [16]. Dilutable nanoemulsions are considered effective drug delivery systems for ophthalmic administration, offering several advantages such as sustained effects and high drug penetration into the deeper layers of ocular structures. Furthermore, cationic nanoemulsions have been identified as superior vehicles for ophthalmic drug delivery. Their interaction with negatively charged corneal cells enhances drug absorption, emphasizing their potential for improving therapeutic outcomes in ophthalmology [65,8].

4. Applications of nanoemulsions in different food sectors

Nanoemulsions are a colloidal particulate system characterised by O/W emulsion properties, with droplet sizes ranging from 10 to 1000 nm and solid spheres with amorphous, lipophilic surfaces [30]. Nanoemulsions, which have fine droplet sizes ranging from 20 to 200 nanometres, have found a wide range of applications in the food industry due to their increased stability and bioavailability. Nanoemulsions can be utilised in the beverage sector to effectively encapsulate bioactive components like vitamins or antioxidants, guaranteeing their effective delivery and retention in functional drinks. Regarding dairy products, nanoemulsions aid in the creation of reduced-fat substitutes that have better creaminess and increased sensory qualities [132]. By enabling greater flavour, colour, and nutrient dispersion and in turn higher product quality, the use of nanoemulsions in the bakery industry is advantageous. Furthermore, the development of condiments and salad dressings benefits from the use of nanoemulsions since they increase the solubility of hydrophobic substances and boost the stability of the final product [154]. In general, nanoemulsions are a promising technology that can be applied widely in the food sector to improve product quality, nutritional delivery, and sensory experiences [117].

The quick transfer of naturally occurring hydrophobic bioactive compounds into the oil droplets is another important function of nanoemulsions. With the further advantages of improved solubility, regulated release, and improved absorption in the gastrointestinal system, this method is useful for encapsulating functional substances like antioxidants and nutraceuticals [51]. Vegetable nanocoatings made from nanoemulsions that contain flavourings, colourings, antioxidants, enzymes, antimicrobials, and anti-browning agents provide a way to extend the shelf life of a variety of food goods, including meats, dairy products, fresh produce, and confections [33,120]. These coatings function as barriers, inhibiting moisture and gas interaction, reducing moisture loss, and slowing oxidation in foods. Biologically active chemicals can have their structures, compositions, and characteristics controlled by means of a nanoemulsion-based method that effectively increases their bioavailability. According to Primožič et al. [110], these nanosized emulsions are used in cheese, meat processing, baking, and food packing. Bioactive substances can be delivered, encapsulated, and protected with the help of nanoemulsions, which have found extensive use in the food sector. The application of nanoemulsions in food sector is briefly reported in Table 1.

4.1. Encapsulation and nutrient delivery

The food sector has shown a great deal of interest in nanoemulsions because they show promise for encapsulation and nutritional delivery. The limited solubility, stability, and bioavailability of bioactives present technological hurdles for the production of functional products. Numerous bioactive food ingredients are vulnerable to oxidative deterioration during storage and degradation during food processing [14]. Some bioactives are volatile and sensitive to processing conditions, while others have limited solubility and quick metabolism [57]. Nanoemulsions provide a way to encapsulate bioactive substances for incorporation into food matrices,

Table 1
Application of nanoemulsions in different areas of food sector.

Application method	Function	Compound / Application	Reference
Encapsulation	To preserve and slow delivery / release of functional compound	Volatile flavors and color agent like; Omega − 3 fatty acids, vitamins and preservatives	Goindi et al. [44]
	To improve the texture and to enhance flavor	β-carotene, curcumin, essential oils, nutraceuticals	Sanni et al. [123]
		Turmeric's yellow colour pigment	Pateiro et al., [104]
		Black pepper essential oil	Vinh, Hien [153]
Preservation	To preserve food quality To enhance antibacterial properties during storage	Bread slices coated with methylcellulose and clove bud essential oil showed a decrease in spoilage molds and a longer shelf-life	Ottoni et al. (2014b)
	To protect against oxidation, reduce loss of moisture, and gaseous exchange	Anise oil coating inhibited foodborne pathogens	Topuz et al. [147]
	To increase shelf life of food products To delay rate of respiration	Thymol coating inhibited growth of mold in cherry tomatoes	Robledo et al. [116]
			[19]
		Nano-emulsified basil oil preserved okra	[46]
		Carvacrol preserved spinach	Zhang et al. [165]
		Nano emulsified fenugreek and Flax seed and essential oils on apple enhanced shelf life	[114]
		Pectin-based covering with nano emulsified essential oil increased the fresh-cut oranges' shelf life	[111]
Packaging	To increase shelf life To maintain the transmission rates	Chitosan and alginate showed a promising packaging material for guava	[11]
		Nano emulsified active packaging prolonged shelf life of chilled chicken fillets	Noori et al. [92]
		Corn nanoemulsions with sodium alginate, and nano emulsions of thyme, lemongrass and mint essential oil for packaging films retarded microbial growth	Severino et al. [129] Artiga-Artigas et al. [12]
New product formulation	To produce novel products with superior sensory qualities and increased stability	Lipophilic vitamins fortified dairy food	Öztürk [100]
		Nisin fortified clear beverages	[3]
	To fortify foods to improve their nutritional profile and give additional health advantages	Nano emulsion fortified coffee, fruit juices, water, soft drinks, and creamy drinks with vitamin D3 and β-carotene	[146] [152] [73]
		Nanoemulsions incorporated bakery products added antioxidant qualities and improved nutritional value	[17] [126]
		Whole grain bread fortified with free lutein	Walker et al. [155]

making it possible to address these difficulties. According to McClements and Rao [81], nanoemulsions improve stability, bioavailability, and regulated release rates by encasing bioactive molecules in an oil phase or emulsifier. Raji et al. [113] have shown the noteworthy effectiveness of these delivery methods when it comes to lipophilic chemicals, nutraceuticals, medicines, flavours, antioxidants, and antibacterial agents. As discussed by McClements [79], nanoemulsions become ideal instruments for enhancing the delivery of functional lipophilic components in the food sector. The delivery system based on nanoemulsions must be able to work with the food matrix while causing the least amount of disturbance to the organoleptic qualities (taste, texture, and appearance). In order to ensure sustained stability against variables including temperature, light, pH, and oxidative conditions during storage, this encapsulation offers protection against processing conditions. For effective use in the food sector, nanoemulsion-based delivery methods must be economically viable for large-scale production. Innovative nano-encapsulation techniques have been developed as a result of the use of nanotechnology to food science. These approaches have advantages include greater surface area, improved stability, improved cellular permeability, and controlled release of bioactive substances [94]. Moreover, Sanni et al. [123] had highlighted the expanding significance of nanoemulsions in delivering desirable sensory qualities to food items, underlining their value as flavour encapsulation systems in the food and beverage sector.

Numerous studies have investigated the nanoencapsulation of bioactive substances such as curcumin [55], quercetin [60], orange peel-derived β-carotene [73], citral [77], and vital nutrients such as major (lipids, carbohydrates, proteins) and minor (minerals, vitamins, essential amino acids, etc.) [15]. Carbohydrate-based nano delivery methods have been investigated in food matrices, including formulations using starch, pectin, cellulose, dextrin, alginate, cyclodextrin, guar gum, and chitosan [39,103]. Pectin–whey protein concentrate complexes were used by Mohammadi et al. [85] to nanoemulsify phenolic compounds from olive leaves. The results showed that the nanoencapsulated compounds had a high degree of stability (96.64 %) and a regulated release (8.1 %) via entire inner droplets conveyance. Polylactic acid-based nanoparticles were used as stabilisers to nano-encapsulate curcumin and quercetin in turmeric extract [139]. These nano-capsules release active substances similar to those found in ordinary meals upon dissolving. Curcumin encapsulated in hydrophobically modified starch demonstrated improved antitumor efficacy [14]. Some substances, such as octenyl succinic anhydride-polylysine, have two functions: they can be surfactants or emulsifiers, and they can be used to encapsulate medicines, antimicrobials, or bioactive substance. Notably, the application of lipid molecules in nanoencapsulation has demonstrated potential in improving antioxidant capacity by means of enhanced bioavailability and solubility, all the while reducing unfavourable interactions with other dietary ingredients. Nanoliposomes, nanocochleates, and archaeosomes are examples of frequently used lipid-based nanoencapsulation systems. Nanoliposomes, for example, act as carriers for nutrients, enzymes, antimicrobials, and additives [52].

The use of single and combined nanoparticles to encapsulate bioactive substances in the food business has been the subject of many research investigations. Using protein nanoparticles to encapsulate hydrophobic and chemically reactive (labile) physiologically active compounds and then combining them with lipid nanoparticles is one significant method. A few research works have centred on creating nanoparticles of zein that are encapsulating tangeritin or filled with curcumin [27,87]. To further enhance the targeted delivery of nutraceuticals such beta-carotene, lycopene, isoflavones, phytosterols, and omega-3 fatty acids, fortifying nanovehicles have been developed [35].

The use of the nanoemulsion approach significantly increased the stability and oral bioavailability of curcumin and epigallocatechin gallate. Additionally, turmeric's yellow colour pigment was enhanced with the use of nanoemulsions [103]. In addition, the process of nano-encapsulating citrus essential oils using nanoemulsions has shown great promise and has important implications in the food industry. As shown by Oprea et al. [94], this highlights the potential of nanoemulsions to efficiently deliver bioactive chemicals inside a variety of food products. Further highlighting the significance of nanoemulsions in the effective delivery of nutrients is the formulation of nanoemulsions with black pepper essential oil, which has been highlighted as a way to improve nutrient absorption in the human body [153]. Zeng et al. [162] showed that nanoemulsions can be used to improve the texture and consistency of ice cream.

Recent advancements in nanoemulsification technology have demonstrated significant potential in targeted nutrient delivery and controlled release within the food industry. Nanotechnology-based nutrient delivery systems can enhance oral bioavailability by stabilizing nutraceuticals within food matrices and improving their solubility in intestinal fluids. For instance, a study by Salvia-Trujillo et al. [121] illustrated the encapsulation of vitamin D within nanoemulsions to fortify orange juice, enhancing its bioavailability without compromising sensory attributes. Similarly, Ozturk et al. [99] reported the successful incorporation of omega-3 fatty acids into yogurt using nanoemulsification, which resulted in improved stability and bioavailability of the fatty acids. Furthermore, Karthik and Anandharamakrishnan (2019) explored the use of curcumin-loaded nanoemulsions in milk, demonstrating improved solubility and absorption of curcumin. The controlled release capabilities of nanoemulsions have been highlighted in a study, where encapsulated probiotics in nanoemulsions exhibited sustained release and enhanced viability in functional beverages. Additionally, Singh et al. [142] developed nanoemulsions for the delivery of essential oils in bakery products, ensuring a prolonged antimicrobial effect and improved shelf life. Ting et al. [146] demonstrated the use of nanoemulsions to fortify beverages with bioactive compounds like vitamin D3 and β-carotene. The nanoemulsions maintained the stability and bioavailability of the nutrients without altering the sensory attributes of the beverages.

4.2. Preservation

Food preservation is one of the numerous fields in which nanoemulsions have attracted attention due to their wide range of uses. Nanoemulsions, especially those containing essential oils, have shown effective in preserving food quality by displaying increased stability and long-lasting antibacterial properties during storage. Fruit quality can be preserved and storage life increased with edible coatings are known to be safe, economical, and efficient [161]. The use of edible nanocoatings based on nanoemulsions could minimise storage-related losses in fresh fruits and vegetables, which are primarily caused by the senescence physical injuries, moisture loss, shrinkage, and microbiological and metabolic degradation. To improve the preservation of food, these nanoemulsions can be used to coat a variety of foods, such as dairy products like meat and cheese, fresh-cut vegetables, fruits, and confectioneries. They may additionally include enzymes, antioxidants, antimicrobial agents, and colour and flavouring ingredients. Food items can be protected against oxidation, reduced loss of moisture, and gaseous exchange by using coatings based on nanoemulsions [33].

Colouring and flavouring compounds are vulnerable to oxidation and photolytic destruction due to the presence of functional groups including aldehydes, ketones, and esters. According to Goindi et al. [44], encasing these components in nanoemulsions prolongs their period of storage by preventing negative effects. Due to being hydrophobic, plant-based essential oils, which are strong in antibacterial capabilities against foodborne microorganisms and viruses, have limitations. This problem can be solved with essential oil-based nanoemulsions, which can also act as natural food preservatives in food sector applications [4]. According to Jo et al. [59], bacterial growth in melon juice was reduced by a cinnamaldehyde-based nanoemulsion with an average particle size of less than 200 nm. Valencia-Chamorro et al. [149] reported that innovative food manufacturing technique that offers a variety of functions i.e. use of edible films and coatings that contain active nanoemulsions. For various fresh-cut fruits like strawberries and pineapple, this method hasn't been thoroughly investigated. According to Gago et al. [41], nanoemulsions that included 1.0 % and 2.0 % w/w cloves and 1.25 % and 2.5 % w/w lemongrass showed enhanced stability and extended antibacterial efficacy, especially when kept at 1 °C, for a period of six months. Gram-positive microbes are generally more susceptible to nanoemulsions than gram-negative microbes, according to literature findings [6]. Donsi [33] stated that the antibacterial capability of nanoemulsions is directly influenced by their physicochemical characteristics, including the size of droplets and electrical charge. Furthermore, the kind of ripening inhibitor used in the production of the nanoemulsion is essential for assessing its antimicrobial efficacy [108].

Several investigations have evaluated the antibacterial and antifungal effects of citrus essential oil-containing nanoemulsions against pathogens and fish deterioration, which is primarily brought on by Gram-negative psychrophilic bacteria. For example, Durmuş et al. [37] used orange, mandarin, grapefruit, and lemon nanoemulsions to increase rainbow trout's shelf life and found that the mandarin and grapefruit nanoemulsions had the greatest effect. Similar to this, Ozogul et al. [98] looked into the antibacterial properties of grapefruit peel essential oil and its nanoemulsions on food pathogens and bacteria that cause fish to spoil. Comparing citrus essential oil nanoemulsions to their natural essential oil equivalents, the antifungal activity of the former showed an improved response [33,76,160]. In a different study, Radi et al. used orange essential oil-containing nanoemulsions and combined them with a pectin-based covering to increase the fresh-cut oranges' shelf life and found that nanoemulsions are more effective than micro-emulsions at fighting microorganisms. Radi et al. [110] reported that microorganisms showed greater susceptibility to nanoemulsion treatment in contrast to yeasts and moulds. Citrus essential oil nanoemulsions demonstrated strong potential for use as flavouring and antimicrobial substances in food items and beverages, with the ability to combat a wide range of common foodborne pathogenic and non-pathogenic microorganisms [62,72]. The effectiveness of nutmeg seed oil-enriched chitosan-based nanoemulsions in maintaining the fresh strawberry quality during a five-day storage period at 10 °C was studied and found effective method to enhance storability by Horison et al., [49]. Robledo et al. [114] assessed the impact of a coating made of quinoa protein/chitosan nanoemulsion and thymol nanoemulsion on the sensory qualities, safety features, and quality of chilled strawberries in a commercial setting. In order to improve microbiological safety and extend the shelf life of grape berries, Kim et al. [63] created nanoemulsion coatings with lemongrass oil (LO). Lemongrass oil based nanoemulsions have been applied to enhance the microbial stability and physicochemical storage of plums and grape berry. The study showed that nanoemulsions of lemongrass oil inhibited the growth of *Escherichia coli* and *Salmonella Typhimurium* which led to enhanced shelf life of fruits [148]. The ethylene production and weight loss of plums was reduced by 4 and 3 folds, respectively [63]. The researchers stated that nanoemulsified lemongrass oil can be utilized for commercial ready-to-eat grape berries to enhance microbiological safety. In one case, fresh-cut Fuji apples were immersed in a nanoemulsion formulation containing alginate-based coatings with lemongrass essential oil for 2 minutes and subsequently stored at 4°C for 14 days. This nanoemulsion coating effectively inhibited the growth of E. coli and the natural flora of the apples for a duration of two weeks [120]. The effects of sodium alginate-based nanoemulsions with different citral levels (1.0 %, 0.5 %, and 0.1 %) on the physicochemical, sensory, and microbiological characteristics of fresh-cut pineapples kept for up to 12 days at 4 °C and 90 % relative humidity were investigated by Prakash et al. [107].

In another study, fresh-water rainbow trout was immersed for 15 minutes in a nanoemulsion formulation that included essential oils from Zataria multiflora Boiss. The treated fish were then packaged in polyethylene bags and stored at 4°C. This treatment not only extended the shelf life of the trout but also preserved its sensory attributes throughout the storage period [131]. According to Rashid et al. [113], the nanoemulsion coatings containing 1.0 g of flaxseed polysaccharide and 1.5 g of fenugreek showed the least amount of microbial degradation. According to Ottoni et al., (2014b), bread slices that were dipped in nanoemulsions containing methylcellulose and clove bud essential oil showed a decrease in spoilage molds and a longer shelf-life. Comparing anise oil-loaded nanoemulsions to their coarse and bulk oil counterparts, Topuz et al. [147] found that anise oil significantly inhibited foodborne pathogens such as *E. Coli* O157:H7 and *L. monocytogenes*. Robledo et al. [116] evaluated the effectiveness of quinoa protein/chitosan nanoemulsions containing thymol in preventing the growth of mould that was inoculated in cherry tomatoes. After seven days, the results showed a notable decrease in the growth of fungi at 5 °C, which extended the fruit's shelf life. These edible coatings significantly increased the hardness of the tomatoes, reduced the overall number of mesophilic bacteria, and prevented the tomatoes from losing three times as much weight as uncoated tomatoes [19]. The potential of alginate coating combined with nano-emulsified basil oil to preserve postharvest quality and stop okra from spoiling was reported [46]. Furthermore, the effects of carvacrol-containing foliar-applied antibacterial nanoemulsion on spinach were studied by Zhang et al. [165]. Carvacrol nanoemulsions have the ability to preserve the postharvest quality of spinach, as demonstrated by the study that found that spraying them at low concentrations (0.005–0.5 %) on spinach leaves preserved the health of the plants.

4.3. Packaging

Nanoemulsions have the ability to be used in the development of film and coating materials for food packaging. Biopolymer-based coatings and films function as the continuous phase that maintains the strength of nanoemulsion [12]. Essential oil nanoemulsion formulations have become a feasible solution to the problems associated with food preservation, and they are being used in natural food preservation as well as food packaging [5].

Edible films featuring nanoemulsions of clove and cinnamon essential oils and antibacterial qualities have been made using pectin, a biopolymer. Since there were changes in the hydrophilic/hydrophobic ratio, pectin films showed low water and vapour permeability [96,125]. Cellulose and its derivatives have been used to produce films with nanoemulsions of oregano and clove essential oils [97]. Severino et al. [127] have utilised chitosan in the production of nanoemulsion coatings and films that include essential oils that possess antibacterial properties, including carvacrol, mandarin oil, bergamot oil, and lemon oil. Corn nanoemulsions that are less size-dispersed and stable have also been created using sodium alginate, along with films that include nanoemulsions of thyme, lemongrass and mint essential oils [2,12].

Films containing rutin-encapsulated soybean oil and canola seed oil and ginger essential oil nanoemulsions have also been produced with antioxidant activity through the application of porcine gelatin [28,5]. Nano emulsified Zataria multiflora essential oil has been incorporated into antimicrobial films using basil seeds [42]. Foods have also been completely absorbed in nanoemulsion formulations to assess their effectiveness as coating agents without the assistance of a matrix of polymers, in addition to the usage of biopolymer matrices. Severino et al. [130] found that the development of *Listeria innocua* was suppressed when green beans coated with a chitosan solution containing nanoemulsions of mandarin essential oil were exposed to UV-C irradiation.

Increased antibacterial activity was demonstrated by nanoemulsions integrated into edible methylcellulose films, such as those made from essential oils of oregano and clove buds, with size of droplets ranging from 180 to 250 nm. By effectively preventing the growth of yeasts and moulds, these nanoemulsion-based films extended the shelf life of bread slices [97]. Similarly, the physical features of active food packaging films improved when ginger essential oil nanoemulsions were included into gelatin-based films [5].

An additional application for curcumin nanoemulsions with a 40 nm average size of droplets was to successfully enhance the shelf life of chilled chicken kept at 4°C for a period of 12 days when they were added to pectin edible coatings. The nanoemulsion demonstrated a variety of the preservation effects, including inhibiting the growth of yeast, moulds, and psychrophiles. It also showed reduced levels for both total volatile nitrogen and thiobarbituric acid, enhanced water holding capacity and texture, and higher sensory scores when compared to the control [1]. In addition, chicken breast fillets were kept fresher longer using nanoemulsions of ginger essential oil coated in sodium caseinate. According to Noori et al. [92], this biologically active coating based on nanoemulsion had more antibacterial activity than antioxidant activity, which effectively inhibited the development of psychrophilic bacteria in chilled chicken fillets over a 12-day period.

Improved bioavailability of lipophilic substances has been demonstrated by nanoemulsions, and edible nanocoatings have been shown to prolong the shelf life of fresh and minimally processed meals. Furthermore, studies have shown that these nanocoatings are effective at enhancing the organoleptic quality of frozen foods when they thaw [75]. Food-grade coatings made of chitosan and alginate show promise as a packaging material for guava [11]. Guava degradation was significantly delayed by the coating's addition of nano zinc oxide, which demonstrated strong antibacterial action. Alginate/chitosan coatings prolonged the storage period up to 20 days under favourable conditions for consumption, a significant improvement over the 7 days of storage for control (uncoated) fruits. Nanoemulsions have been studied for their possible use in active packaging and show biodegradable qualities [109].

4.4. New product formulation

Nanoemulsions are widely acknowledged as a novel substance that can be utilised to produce novel products with superior sensory qualities and increased stability. Nanoemulsions are becoming more prevalent in the fortification of many foods to improve their nutritional profile and give additional health advantages. Probiotics, vitamins, and minerals can be incorporated into dairy products including ice cream, cheese, and yoghurt using nanoemulsions. Nanoemulsions have been used to add vitamins, and essential fatty acids to savoury foods such as sauces, dressings, and condiments. Also, to improve the nutritional value of functional meals like granola, energy bars, and cereal goods, nanoemulsions have been added. As the food industry becomes more interested in using edible nanoemulsions to encapsulate and distribute lipophilic functional components, the use of nanoemulsions in food fortification becomes reasonable [74]. According to Mehmood and Ahmed [83], their efficacy also extends to the food and beverage industry when it comes to integrating β-carotene.

For the purpose of adding bioactive substances to dairy products, nanoemulsions have become a promising method. Nanoemulsions have been shown to enhance dairy desserts with angelica essential oil (AEO) [90], lutein extract [118], and omega-3 fatty acids [156]. The stability, bioavailability, and physicochemical characteristics of the fortified dairy products have all been demonstrated to be improved by these nanoemulsions. Furthermore, lipophilic vitamins in fortified meals have been suggested to be effectively delivered using nanoemulsions [100]. Because nanoemulsions can encapsulate and distribute antimicrobial compounds, such nisin, in clear and fortified beverages, their use in the food and beverage industry is further strengthened by this capability [3].

Research has shown that nanoemulsions work well in a wide range of beverages, including coffee, fruit juices, fortified water, soft drinks, and creamy drinks [146]. Additionally, beverages can be enhanced with bioactive substances such as vitamin D3 and β-carotene through the use of nanoemulsions, which maintain sensory qualities and increase the stability and bioavailability of the beverage [146,152]. It has been demonstrated that incorporating nanoemulsions to bakery products increases the stability and bioavailability of bioactive compounds, adding antioxidant qualities and improving nutritional value, and assisting in the creation of functionally fortified bakery products [17,126].

The potential of nanoemulsions to fortify and deliver omega-3 fatty acids in bakery products has also been investigated, providing insight into the opportunities and difficulties faced by the food sector in supplying these essential fats [156]. Additionally, research has examined the stability of lutein in wholegrain bread items that are either naturally high in lutein or have been fortified with free lutein, indicating the possibility of adding bioactive substances to bakery products to enhance their fortification. Omega-3 fatty acids may be effectively added to liquid food systems including sauces and beverages using nanoemulsions, which highlights the promise of this technology for fortifying a variety of food items.

4.5. Commercial applications in food industry

In this section, successful commercial applications of nanoemulsion based products in food industry are highlighted to provide practical insights. Nano-sized self-assembled structured liquids developed by Nutrelease has been utilized by many industries to fortify food with functional compounds like omega-3 fatty acids, pigments etc. especially beverages and oil to ensure preservation and maintain quality. Aquanova has developed NovaSol beverages, incorporating nanoemulsions of various functional compounds and natural colorants, including β-carotene, chlorophyll, sweet pepper extract, lutein and curcumin. The encapsulated compounds in NovaSol beverages exhibit enhanced stability and consistent additive concentrations [9]. Similarly, Shemen Industries utilize nanostructured liquids to produce Canola Active oil, which is enriched with non-esterified phytosterols. These nanoemulsions facilitate the competition of phytosterols with cholesterol for incorporation into micelles, thus reducing cholesterol absorption from the digestive tract. Prominent food industry players such as Nestlé has developed and patented nanoemulsions using polysorbates and micelle-forming emulsions to facilitate rapid and uniform thawing of frozen foods in microwaves in their products [121]. Nestlé and PepsiCo have also developed clear beverages fortified with vitamin D using nanoemulsion technology. The nanoemulsions ensure that vitamin D is stable and bioavailable in a clear, transparent liquid matrix. Unilever has also incorporated nanoemulsions into its ice cream products and salad dressings to reduce fat content. Shenzhen Become Industry & Trade Co., Ltd. has developed a product called Nanotea, which features nano fine powder with claimed antimicrobial properties and a tenfold increase in selenium content. Bottled waters enhanced with electrolytes, flavors (soluble in oil), and vitamins through nanoemulsion technology have been introduced, demonstrating the versatility of nanoemulsions in functional beverage applications [104].

Flavour nanoemulsions have been utilized to create stable and optically clear liquid beverages, including alcoholic drinks [67]. Patents have been filed for the use of nanoemulsified non-ionic surfactants to enhance the delivery of hydrophobic food flavourings [158,93]. Nicolosi & Wilson (2018) patented a premix formulation combining an oil-based medium with an aqueous component to improve the bioavailability of incorporated compounds. Additionally, Huang (2019) developed and patented a nanoemulsified formulation of cyclosporine to enhance its delivery across various applications, including food. Patents have been granted for nanoemulsions containing natural antioxidant used in food preservation. These nanoemulsions, which encapsulate natural antioxidants and are freeze-dried, have been employed to extend the shelf life of fresh and minimally processed foods. Additionally, they have been shown to enhance the sensory quality of frozen foods [75]. RBC Life sciences has developed Nanoceuticals with cocoa and without sugar, showed the monitored delivery of nutrients with antioxidant properties.

5. Gastrointestinal fate of nanoemulsions

After ingestion, nanoemulsions are exposed to a complex physiological environment as they pass through the digestive tract where they undergo both compositional and structural changes. There is a growing interest in designing nanoemulsions intended for targeting delivery of bioactive agents to different regions (the mouth, stomach, small intestine, and colon) of the gastrointestinal tract (GIT). Mouth is the first region that a delivery system encounters after consumption, where the food interacts with saliva within the oral cavity. Saliva contains various biopolymers (such as mucin) and enzymes (such as amylase). Long protein chains with charged oligosaccharide moieties of mucin can easily interact with the food matrix, thus affecting the release of bioactives and flavors. Amylase may hydrolyse any starch present and other environmental conditions within the mouth, such as pH, ionic composition, and temperature, may trigger the breakdown of the matrix surrounding the oil droplets and thereby promote their release. The bolus arising from the oral phase travels down the esophagus and goes to the gastric chamber. Alterations in the pH and ionic composition of the gastric fluids within the stomach may have a major impact on the stability of nanoemulsions because of their influence on the electrostatic interactions between the oil droplets and other charged components in the system. The stability of a nanoemulsion to gastric conditions is therefore highly sensitive to the nature of the emulsifier molecules that coat the droplets. This might result in instability due to chemical degradation of some components e.g., the hydrolysis of adsorbed protein by pepsin or the oxidation of polyunsaturated lipids by lipase [164]. The fluids resulting from the stomach (chyme) then enter the small intestine, which is the region where most of macronutrients and micronutrients are digested and/or absorbed. The nutrient absorption is facilitated by the complex mixture of digestive enzymes, coenzymes, phospholipids, bile salts, and bicarbonate salts, and other minerals present in intestinal fluid. Colon is the final part of GIT and has a major function in fermenting any indigestible components (such as dietary fibers) that have passed undigested through the upper GIT. It may be desirable to develop nanoemulsion-based systems that can deliver specific components to the colon. The fermentation products (such as short-chain fatty acids) stimulate the preferential growth of colonic bacteria that may be beneficial to human health.

The gastrointestinal fate of the emulsion-based delivery systems is examined through in-vitro and in-vivo testing. It is critical that nanoemulsion based system should withstand the stresses within the GIT in order to provide bioaccessibility after consumption.

5.1. *In-vitro gastrointestinal studies*

This is based on using simulated gastrointestinal models to understand how nanoemulsion behave inside the human gut, the rate and extent of macronutrient digestion, and the bioaccessibility of encapsulated bioactive substances. The static in-vitro digestion method developed by the INFOGEST international consortium is the most widely applied model. This model is designed to provide instructions on the conditions that should be applied in each region of the GIT, including temperatures, pH values, incubation times, mineral compositions, bile salts concentrations, mucin levels, enzyme activities, and mechanical forces. Dynamic models are also most common to simulate real system more accurately where the conditions in each gastrointestinal region are varied over time [36]. In-vitro digestion model may also be used to provide evidence about the chemical or biochemical transformation of encapsulated components within the GIT (such as oxidation or hydrolysis). Occasionally, they have been extended to deliver insights into the absorption of bioactives through the epithelium cells lining the small intestine [78].

5.2. *In-vivo gastrointestinal studies*

A more accurate understanding can often be obtained using animal feeding studies through oral administration by injecting it directly into the animal's stomach. The GIT fate can then be monitored by sacrificing the animal and measuring the concentration of the administered bioactive components in different regions of GIT, bloodstream and specific organs (such as the liver, kidney, heart, adipose tissue, muscles, intestinal tissue, brain, etc.). Pharmacokinetic experiments can also be carried out using human feeding studies, provided the formulation is known to be safe and appropriate approval has been obtained. In one study, it was shown that nanoemulsions improved the oral bioavailability of ω-3 fatty acids in humans after they were incorporated into yogurts, when compared to a bulk oil [66]. In another human feeding study, it was shown that emulsified cod liver oil had a significantly higher bioavailability than bulk cod liver oil [26].

6. Safety and toxicological analysis

The safety and toxicity of nanoemulsions depends on their interactions with biological systems. The small size and large surface area of nanoparticles allow them to easily pass through tissue and cell barriers, which may have a negative impact on biological systems. Beside the size and shape of nanoparticle its charge, digestability it may influence the potential toxicity. Many studies have shown increased bioavailability of nanoemulsion which may promote adverse health effect in certain population with excessive consumption of nanoemulsion for few substances. Another potential source of toxicity is related to the type and amount of ingredients used to formulate nanoemulsions. As higher emulsifier levels are required to stabilize their higher surface areas and, in some cases, the small droplet sizes can only be created using small molecule synthetic surfactants, which may be more toxic than natural emulsifiers. As regards with the safety, a precise selection of the initial materials for the oil phase, aqueous phase, surfactants and co-surfactants are required. The administration route may also affect the toxicity of nanoemulsion. This toxicity problem can be overcome by formulating nanoemulsions using only those ingredients known to be safe when used at the required levels.

A number of researches have carried out in-vitro to assess the potential toxicity of the nanoemulsion. The in-vitro study also includes oxidative stress, DNA damage, and accumulation or localization of NPs which may be evaluated using electron microscopy. The potential cytotoxicity of unloaded and curcumin-loaded nanoemulsions was tested and both the nanoemulsions showed no toxicity against the mouse fibroblast. Also, the curcumin loaded nanoemulsions did exhibit strong cytotoxicity against colon cancer cell lines [151]. Apart from the cytotoxicity, genotoxicity and carcinogenicity are some possible adverse effects of nanoparticles. This cell viability can be assessed by EZ4U, MTT, MTS, clonogenic assay etc. for in-vivo analysis on different cell lines of human, rat or bacteria (for antimicrobial study) etc. Similarly, genotoxicity study involves comet assay, micronucleus, tetrazolium reduction assays etc.

Kaur et al. [61] found no toxicity in Hep G2 cells in nanoemulsion based on D-α-tocopherol, lemon oil, tocopheryl polyethylene glycol succinate (TPGS), tween 80. Using TPGS as an emulsifier, Han et al. [48] observed no toxic effect on buffalo rat liver cells (BRL-3A) cells for capsaicin nanoemulsion when its concentration was at or below 3 μg mL^{-1}. While cell viability dropped to 83 % at 15 μg mL^{-1}. The toxicity evaluation in human hepatic cell line (WRL-68) indicated no significant toxicity of fenugreek oil nanoemlusion upto the concentration of 800 μg/mL. A study conducted on nanoemulsions examined their toxicity in Caco 2 cells. The results indicated that the toxicity of these nanoemulsions increased when β-carotene was loaded into them [158]. This was attributed to the formation of reactive oxidative species inside the cell due to oxidation and metabolism of β-carotene. Additionally, they found increased cytotoxicity for Tween 80-stabilized nanoemulsions with increasing droplet diameter particularly at high doses. Overall, the authors suggested that the toxicity of the nanoemulsions was not due to their small size but due to inclusion of specific ingredients (such as surfactants, alcohols, and carotenoids). Based on the findings, it suggests that nanoemulsions generally do not have strong cytotoxic effects, as long as they are made with ingredients that are safe for consumption. However, in-vitro tests fail to provide reliable data about biological life, bioavailability and other pharmacokinetic aspects of a nanoemuslion, thus in-vivo analysis is suggested to assess the potential toxicity of nanoemulsion. Essential oil nanoemulsion of *Cinnamomum travancorium* showed anti-diabetic activity and no toxicity in in-vivo study [144]. Silva et al. [136] studied in-vivo toxicity of goldenberry extract nanoemulsion using *Caenorhabditis elegans*, a free-living nematode using Escherichia coli as food source and found no toxic effects.

Most of the literatures aforementioned on the safety and toxicity of nanoemulsions in biological systems suggest that their application is safe. There are few studies where researchers have found that nanoemulsions are toxic probably because of the very high doses employed. Few studies on the interactions of nanoemulsion droplets with biological systems are provided in Table 2. Hort et al. [50] treated Male Wistar rats once daily for 21 days with nanoemulsion via oral or intraperitoneal delivery at 200, 400 or 800 mg lipid/kg body weight and analyzed biochemical, hematological, oxidative stress, and genotoxicity parameters. The organ weight or biochemical parameters were not modified compared to controls. The highest dose (800 mg/kg) via intraperitoneal injection caused some inflammatory reactions like, changes in hematological parameters, namely increased plasma proteins, platelets, total leukocytes, and neutrophils.

There is a wealth of literature available on in-vivo testing for drug delivery in the pharmaceutical field. However, there is still a lack of information regarding the potential toxicity of nanoemulsions in-vivo in the food sector, which should be investigated before carrying out human clinical trials. It is important to consider the route of administration while discussing the toxicity of nanoemusion. Typically, the food-based nanoemulsion needs to be administered orally.

7. Regulatory aspects and public perceptions

There are some comprehensive studies on nanomaterial and its toxicity, but there is a lack of common regulatory guideline available to address potential risks involved in it Katouzian and Jafari, 2016 [105]. While certain nanomaterials used in foods may pose risks, it is crucial to note that all should not be considered potential hazards [31]. It is essential for each country to establish an organization responsible for regulating and assessing the risks associated with nanotechnology. The regulation of nanoscale products is still in its development stages and there is no global regulatory authority to develop robust standardized procedures for their proper physicochemical characterization, risk assessment, and exposure assessment for nanoemulsions. Although there is no separate regulation for this, researchers follow the regulation that are applicable to nanomaterials in general.

In the European Union, the European Food Safety Authority (ESFA) includes the nanomaterials under the regulatory framework that ensures safe use of all chemicals and mixtures. To be legally manufactured or imported in the EU, all substances have to be registered. As part of their registration, manufactures and/or importers must submit information on both human health and environmental effects, and hazardous nanoforms – an estimation of exposure throughout the life cycle. substances have hazardous

Table 2
Impact of nanoemulsions on the biological systems through in-vivo studies.

Study	Bioactive	Surfactant	Subject/Cell line	Results	References
Toxicity	*Eucalyptus staigeriana* essential oil	Tween 80	Female Wistar albino rats	Ovicidal and larvicidal effects on H. contortus. No hematologic alterations in rats	Ribeiro et al. [115]
Safety against nephrotoxicity	Paclitaxel	Tween 20	Swiss albino mice	No hematologic, biochemical, or structural toxicity at 6.5 and 3 mg/kg oral doses. Decrease in RBC, hemoglobin, and neutrophil counts at 12.8 mg/kg	Choudhury et al. [24]
Antiglioma, cytotoxicity, and genotoxicity oil	Pomegranate seed	Tween 80	Peripheral human blood. Rat glioma cell line (C6)	Increased cell viability with no genotoxicity and oxidative damage Low toxicity against human blood cells at 0.05 mg/mL	Ferreira et al. [86]
Dyslipidemia	Garlic oil	Tween 80	Wistar rats	No hematologic or histological changes at 0.46 mL/kg dosage. Immediate mortality at 18.63 mL/kg	Ragavan et al. [112]

properties, the Classification, Labelling and Packaging Regulation (CLP) requires them to be notified and labelled and packaged so the substances can be used safely. Companies should to be transparent in their registration to clearly indicate how the safety of nanoforms has been addressed, including what measures are needed to adequately control the potential risk. Upper particle size limit for nanofoods and ultrafine foods may be loosened to a value above 100 nm depending on the product types.

In the United States, the Food and Drug Administration (FDA) oversees the authorization of food additives, including those involving nanotechnology. Nanotechnology-based agricultural products are also regulated by the Environmental Protection Agency (EPA) and the United States Department of Agriculture (USDA). The FDA has addressed nanotechnology-related issues in publications such as "Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology". Other countries like Canada, Switzerland, Japan, and China also have agencies responsible for reviewing nanotechnology issues in food.

In India, the regulation of nanotechnology-based agri-products falls under the purview of various agencies and laws, including the Department of Biotechnology (DBT), the Ministry of Environment, Forest and Climate Change (MoEFCC), the Food Safety and Standards Authority of India (FSSAI), and the Indian Council of Agricultural Research (ICAR). Existing legislation often forms the basis for regulating nanomaterials, but challenges persist in aligning nanotechnology legislation with industrial applications [7]. Although relevant legislation is still evolving, actions can be taken to ensure consumer acceptance of foods containing nanotechnology-based materials. This involves using food-grade ingredients to produce nanosystems [141].

With the continuous development of food nanotechnology, the safety of all new products needs to be verified, and developers and regulatory agencies should have sufficient evidence to prove the safety of product before it can be marketed. This lack of shared awareness has led to unfamiliarity and uncertainty among consumers regarding nanotechnology or nanoemulsion. Public perception and knowledge about nanotechnology differ depending on the surveyed region and it is also changing over time [45]. The study done on consumer perception towards nanotechnology by Feindt and Poortvliet [40] and Kuang et al. [64] presented that the consumers are willing to buy the nanotechnology foods, when they are aware of its functional benefit and the belief that the new technologies and the product would not bring negative health effects. These results also showed that a majority of young educated consumers had positive attitude towards nano-food. Social influence also has a direct effect on attitude towards such foods and trial willingness. Increasing public awareness about the advantages of nanoemulsion through various educational programs and information campaign can help address any negative perceptions. It is also important that the food industry provide consumers with trustable, understandable and clear information regarding nanoemulsions, including their advantages and safety precautions, through food labelling. Additionally, the recommended daily allowance (RDA) and tolerable upper intake level (UL) need to be re-examined and re-evaluated. With summing up, toxicity analysis must be inevitable before any human applications with all regulatory requirements assessed by utilizing internationally agreed free of bias in-vivo toxicological models.

8. Conclusions and future directions of research and development

Nanoemulsions have potential advantages of nanoemulsions over traditional emulsions, including enhanced solubility, bioavailability, and improved physical stability, make them ideal carriers for delivering active ingredients and thus, present a promising approach for pharmaceuticals and sustainable food processing. Significant advancements have been made in the formulation, production methods that providing opportunities for the various applications. Numerous studies have demonstrated the usefulness of nanoemulsification by bioactive characterization and in-vitro studies. There are also literatures that show the actual health benefits through in-vivo testing on animal and human models. Nevertheless, incorporation of nanoemulsions containing bioactive compounds into commercial food products is still very limited. Studies have suggested that nanoemulsions can be considered safe for oral administration, but high doses via the parenteral route can cause toxic effects. Due to the lack of information on toxicological effects on food, until now, ambiguities on consuming nano-scale food materials still exist. The potential toxicological effects, safety and biological fate of nanoparticles after digestion need to be elucidated and further, risk analysis has to be conducted particularly concerning long term exposure and accumulation in biological system. Several regulatory challenges are there which must be addressed to facilitate their widespread applications. Improvements in regulatory systems are important also in order to prevent consumer misinformation and change the perception. These are crucial to set some standards of consumption limits to be included in labelling which will help in changing public perception and enhance awareness. The application of such nanomaterials for foods requires that the technique is economically feasible for industrial scale production. Extensive research is required in these directions especially examining the scalability and commercialization potential of nanoemulsion incorporated systems.

CRediT authorship contribution statement

Chirasmita Panigrahi: Writing – review & editing, Writing – original draft, Conceptualization. **Anjali Khuntia:** Writing – review & editing, Writing – original draft. **Monalisa Sahoo:** Writing – review & editing, Visualization, Supervision, Conceptualization. **Gurveer Kaur:** Writing – review & editing, Writing – original draft. **Swati Agarwal:** Writing – review & editing, Writing – original draft, Investigation.

Declaration of Competing Interest

The authors declare that the authors have no competing interests, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

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