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Nanoemulsions: stability and physical properties

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Nanoemulsions present several advantages over conventional emulsions due to the small droplets size they contain: high optical clarity, good physical stability against gravitational separation and droplet aggregation, and enhanced bioavailability of encapsulated substances, which make them suitable for food applications. Depending on desired formulation, preparation method should be selected to optimize droplet size distribution since it strongly affects stability behavior. Systems with droplets diameter smaller than 200 nm and a monomodal distribution usually have a homogeneous structure, that is, a structure with well-distributed droplets that do not show flocs. A structure with these characteristics remains unchanged for long time, up to six months, given the nanoemulsion enhanced stability compared to conventional emulsions with the same formulation.

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Introduction

In the last several years, food nanotechnology is gaining more interest from both scientific and industrial points of view. Nanotechnology can be applied to all areas of food science such as food processing, packaging, safety, nutrition and nutraceuticals [1]. Nanoemulsions are defined as a thermodynamically unstable colloidal dispersion consisting of two immiscible liquids, with one of the liquids being dispersed as small spherical droplets with radius sizes smaller than 100 nm [2]. A conventional emulsion typically has particles with mean radii between 100 nm and 100 μm [3]. Both conventional emulsions and nanoemulsions are metastable systems, meaning they have a tendency to break down over time due to a variety of destabilization mechanisms, such as gravitational separation, coalescence, flocculation, and Ostwald ripening [4•]. However, emulsions may be formulated to remain stable for a desired period. Addition of stabilizers and co-adjuvant molecules may have important effects on physical properties and stability of nanoemulsions since molecular interactions strongly influence structure and rheological behavior.

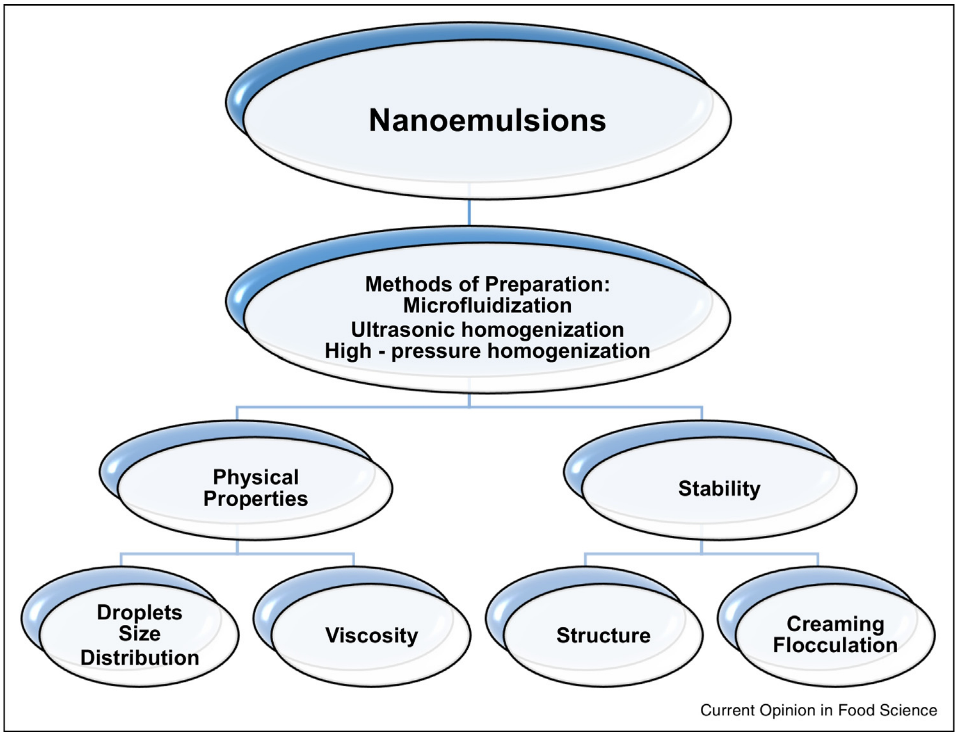
Nanoemulsions may present several advantages over conventional emulsions due to the small droplets size they contain. Some of these advantages are high optical clarity, good physical stability against gravitational separation and droplet aggregation, and enhanced bioavailability of encapsulated substances, which make them suitable for food applications. In the beverage industry, high transparency is a very favorable and appealing characteristic of nanoemulsions since the small droplets size ensures a weak light scattering of final products; therefore, addition of functional ingredients encapsulated in a nanoemulsion will not visibly alter the appearance of a final beverage product [5]. In delivery of nutraceuticals, vitamins, drugs, antimicrobials, colors or flavors, the physicochemical and structural properties of nanoparticles formed must be controlled regarding the desired application [6,7]. Food protein stabilized nanoemulsions have been studied as delivery systems because they have excellent emulsifying properties, good binding capacity for hydrophobic bioactive compounds, and excellent gelation properties. The most common proteins used in emulsion stabilization are dairy-based proteins such as casein and whey proteins. Plant proteins (e.g., proteins from soy, pea, lentil, canola) are less understood as emulsifiers. However, in recent years there has been a growing interest toward utilizing plant proteins [8•].

Many articles deal with scientific evidence confirming that nanoemulsions have enhanced properties compared to similar non-nano-formulated delivery systems [9–14], to name a few. The aim of this review is to summarize the most relevant contributions reported in the last two years addressing the study of nanoemulsions physical properties and stability. A scheme of content is shown in Figure 1.

Methods of preparation

Emulsions may be produced by two methods: high-energy and low-energy emulsification. The methods

Figure 1



Selecting an appropriate method to prepare nanoemulsions allows obtaining small droplet sizes and a monomodal distribution, which results in an homogeneous structure that leads to stability to creaming and flocculation.

are classified based on the physicochemical mechanisms involved. In high-energy methods, droplet disruption is mainly achieved by generating large pressure differences within mechanical devices or ultrasound waves [15•]. These methods are preferred in industrial operations because of the flexible control of emulsion droplet size distribution and ability to produce fine emulsions from a wide variety of materials. As they are easier to scale up than low-energy methods, the examples selected in Table 1 belong to the high-energy preparation group. The most common methods are microfluidization [16,17], high-pressure homogenization [18], and ultrasonic homogenization [19]. Microfluidization is a widely used technique for producing nanoemulsions. The conventional microfluidizer device uses a pump to force a coarse emulsion pre-mix to a chamber under high pressure. Thus, microfluidization is a two-step process that depending on formulation leads to emulsions with droplet sizes smaller than 600 nm. Generally, mean particle size tend to be smaller when synthetic surfactants are used rather than natural ones. For natural emulsifiers, smaller droplets sizes may be obtained by using a dual channel

Table 1

Average diameter of droplets (*Z*-average) for food grade nanoemulsions produced by different high-energy homogenization methods.

System	Homogenization method	*Z*-average (nm)	Reference
Pea protein isolate-sodium caseinate/corn oil	High-pressure homogenization	140–180	[8•]
Sodium caseinate/medium chain TAGs/butanol	High-pressure homogenization	<150	[15•]
Bovine serum albumin/corn oil/poly ethylene glycol	Ultrasonic emulsification	≈100	[27•]
Citral/span 85-Brij 97/ethylene glycol	Ultrasonic emulsification	<100	[28]
Essential oil/alginate	Microfluidization (Two step method)	2–21	[32]
Carotenoid/linseed oil/Tween 20	High-pressure homogenization	<200	[33]
Sodium caseinate/DHA; Tween 40/DHA; Soya lecithin/DHA	Microfluidization (Two step method)	206–148–760	[35•]
Kenaf seed oil/Tween 20	High-pressure homogenization	≈100	[36]
Wheat bran oil/Span 80-Tween 80	Ultrasonic emulsification	10–100	[38]
Ergocalciferol-soy bean oil/modified lecithin	High-pressure homogenization	126–138	[39]
Flaxseed and algae oils/Tween 40	Ultrasonic emulsification	200–250	[40]
Medium-chain triglyceride oil/soy lecithin or Tween 20	Ultrasonic emulsification	140–230	[47]

microfluidization method. In this method, oil and aqueous phases are placed into the microfluidazer separately. That is, the premix step is not required in this one-step method [16]. According to Bai *et al.* [16], droplets coated by some natural emulsifiers are more difficult to break down within a homogenizer because they give a higher interfacial tension and elasticity. Molecular rearrangements and cross-linking of emulsifiers may occur at the droplet surface after the preparation of the initial coarse emulsion and before the homogenization step through the microfluidizer. These rearrangements may reduce the efficiency of droplet disruption during homogenization [16]. For these reasons, the dual channel microfluidization homogenization is a better method than the two-step process to prepare nanoemulsions stabilized with natural emulsifiers [17].

Ultrasonic homogenization and high-pressure homogenization are also two-step processes. For the latter, high-pressure valve homogenizers are currently the most widely utilized mechanical devices for producing emulsions in the food industry [18]. For the former, driving force is the local pressure field within the system [19]. The device usually used for ultrasound process has a tip of a rod or piston that oscillates within a fluid at typical frequencies in the range of 20–40 kHz. Microfluidization, ultrasonication or high-pressure homogenization do not usually lead to nanoemulsions when the selected stabilizer is a protein. Larger quantities of a protein stabilizer are needed to obtain a nanoemulsion compared to the amounts needed for small molecule emulsifiers. Also in some cases, formulations need to be more complex to obtain smaller droplets. Low energy methods, however, usually lead to stable nano systems, but they are only applicable to certain combinations of oils and emulsifiers [4•,20]. Low energy approaches utilize the intrinsic properties of the emulsifier, oil, and water systems to form nanoemulsions. During preparation, spontaneous formation of small oil droplets occurs at the boundary between the aqueous and organic phases under certain system conditions. The most used methods are phase inversion composition (PIC), phase inversion temperature (PIT), and spontaneous emulsification. During the PIC method, an emulsion is formed when water is added to an oil–surfactant mixture. The surfactant must have high affinity for water. The method uses the chemical energy released from the emulsification process as a consequence of change in the spontaneous curvature of surfactant molecules from negative to positive (obtaining oil-in-water nanoemulsions) or from positive to negative (obtaining water-in-oil nanoemulsions). The PIT method relies on changes in the solubility of non-ionic surfactants, resulting from changes in temperature. At low temperatures, the headgroups of the non-ionic surfactants are highly hydrated, which favor the formation of oil-in-water nanoemulsions. As temperature increases, these headgroups become progressively dehydrated favoring the formation of water-in-oil nanoemulsions [21,22]. Spontaneous emulsification consists of mixing the dispersed phase with a surfactant with high affinity toward the continuous phase and then adding the homogeneous mixture to the continuous phase. The main disadvantage of low-energy approaches is that high levels of synthetic surfactant are often required [23,24]. Both, low and high energy methods may be improved by dissolving the oil phase in a solvent and adding an evaporative step [25]. This combination of methods is especially successful for protein-stabilized emulsions [26].

Physical properties and stability

Table 1 reports average diameter of droplets (*Z*-average) of food grade nanoemulsions produced by different high energy homogenization methods. Depending on the system under study, the same method may allow preparation of nano or conventional emulsions. The selected systems are successful examples of nanoemulsions or examples of conventional systems that became nano by addition of a co-adjuvant compound. This is the case of bovine serum albumin/corn oil-in-water (O/W) emulsions. When they were prepared homogenizing coarse emulsions with an ultrasonicator, formulations that contained 20 wt.% corn oil and 15 wt.% bovine serum albumin always had mean droplets diameters greater than 500 nm. Although emulsions were prepared using different sonication times conventional emulsion were obtained in all conditions selected. However, the nano range was achieved through changes in the emulsion formulation by adding 20 wt.% polyethylene glycol [27•]. Polyethylene glycol improved solvent performance acting as co-adjuvant. Other compounds, such as ethylene glycol, were also used as co-solvent. Citral nanoemulsions with average droplet sizes below 100 nm were successfully prepared for delivery purposes [28,29]. Protein-stabilized systems, such as sodium caseinate emulsions prepared in a high-pressure homogenizer, also became nano by addition of a co-adjuvant such as butanol [15•]. A probable explanation is that emulsions prepared with low viscosity oils contained considerably smaller droplets than those made with high viscosity oils. As butanol decreased systems viscosity, droplet sizes fell into the nano range. Zeeb *et al.*'s study [15•] reported that the droplet size decreased as the alcohol concentration was increased up to a certain level (≈10 wt.%), which was attributed to a reduction in interfacial tension. However, the droplet size then increased upon further alcohol addition, due to the effect of alcohol on droplet flocculation. Therefore, alcohol enhanced solvent performance at an optimum concentration interval. Other co-adjuvants such as eugenol were also useful to obtain more stable nanoemulsions using natural stabilizers [30,31].

It is easier to obtain droplets in the nano range when systems are stabilized by small-molecules emulsifiers. For example, the microfludization method was successfully used to prepare nanoemulsions containing essential oils (lemongrass, clove, tea tree, thyme, geranium, marjoram, palmarosa, rosewood, sage or mint) stabilized by Tween 80 and sodium alginate. Compared to coarse emulsions prepared by high shear homogenization, nanoemulsions viscosity decreased with at least 30% drop in their initial values and all of them looked transparent [32]. Another example of stable nanoemulsions prepared using a small molecule stabilizer (Tween 20) was reported by Sotomayor Gerding *et al.* [33]. These authors used high-pressure homogenization to obtained caretonoid/linseed oil nanoemulsions. Astaxanthin or lycopene in different concentrations were emulsified using different processing conditions (homogenization pressures of 5, 10, and 100 MPa) and stability of systems was evaluated at various processing environments (different temperatures, pHs, and NaCl concentrations). When homogenization pressure was 100 MPa nanoemulsions had droplets diameters below 200 nm. For other pressures, emulsions had droplets sizes within conventional emulsions range. Both examples show the relevance of emulsion composition and processing conditions on physical properties and stability. Droplet size distribution depends on formulation and processing parameters [34], and it strongly influences microstructure and stability behavior. In addition to these factors, environmental factors are very relevant to stability. For example, carotenoids nanoemulsions were unstable at acid pH due to degradation of protonated carotenoids [10,33]. With the aim of comparing the performance of different types of emulsifiers, Karthik and Anandharamakrishnan [35•] prepared docosahexaenoic acid (DHA) oil-in-water nanoemulsions by microfluidization. To stabilize the systems they used three different types of emulsifiers: non-ionic (Tween 40), ionic (sodium caseinate) and amphiphilic (soya lecithin). Tween 40 nanoemulsions had the lowest mean diameter of the three formulations and its structural characteristics as evaluated by confocal microscopy showed no changes during storage. Nanoemulsions stabilized by sodium caseinate resulted in flocculation and coalescence. Results from this work clearly show the challenges associated with preparing stable nanoemulsion using proteins as emulsifiers. Another reported example is the study of Cheong *et al.* [36]. In their work, nanoemulsions were prepared with 10 wt.% kenaf seed oil, stabilized with Tween 20, and homogenized by a high-pressure approach. The systems were stable with droplets size in the nano range. However, when sodium caseinate was added to this formulation, the resulting emulsion was in the conventional range [36]. A mixed interface of Tween 20 and sodium caseinate behaved as a poor stabilizer probably due to competition of Tween 20 and sodium caseinate molecules at the interface. However, stable sodium caseinate-stabilized nanoemulsions were prepared by using the same high-pressure homogenizer method. In this case, the stabilizer was a mix of two proteins from different origin: sodium caseinate (a milk protein) and pea protein isolate (a plant protein). Interactions between both proteins were positive, that is, they were stronger between sodium caseinate and pea protein molecules than between molecules of the same protein. The presence of both proteins in formulation prevented flocculation and nanoemulsions were stable for 6 months [37••]. This example shows the relevance of molecule interactions in stability. When interactions are negative nanoemulsions may destabilize depending on their droplet sizes by creaming or flocculation [38,39]. Big droplets are very much affected by gravity and the presence of big droplets usually leads to creaming. When interactions are positive, however, homogeneous structures that do not show flocs or big droplets are always reported [40–44]. The well-distributed small droplets systems are stable for long times [37••,45–47]. Therefore, the challenge is to find a formulation with positive interactions among components and a method to obtain particles in the nano range. Selecting the right environmental conditions, the resulting system will be stable for months.

Conclusions

To produce stable nanoemulsions several factors such as emulsion composition, homogenization conditions, and environment must be optimized. Regarding composition, emulsifier type and concentration, oil type, and co-solvent addition are of great importance. When selecting processing conditions, homogenizer type, pressure, number of cycles, steps involved in preparation are also very relevant. Other factors such as temperature, pH, and salts concentration strongly influenced physical properties and stability. Protein-stabilized nanoemulsions are more difficult to prepare than small molecule-stabilized systems. For a selected system, the best conditions are the ones that minimize droplets size since nanodroplets systems are more transparent than conventional systems and have a more homogeneous structure that lead to higher stability.

Conflicts of interest

The authors declare no conflicts of interest.

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