Chapter

Encapsulation of Natural Bioactive Compounds: Nanoemulsion Formulation to Enhance Essential Oils Activities

Mariem Ben Jemaa, Hanen Falleh and Riadh Ksouri

Abstract

The microencapsulation technology consists of a trap of a compound inside a tiny sphere known as microsphere. The microencapsulation concerns many different active materials such as bioactive compounds, drugs, vitamins, enzymes, flavors, and pesticides. This technology has gained real interest in numerous fields such as agriculture, cosmetic, pharmaceutical, textile, and food. This chapter highlights the encapsulation of essential oils into nanoemulsion-based delivery system as a model for the encapsulation of natural bioactive compounds. Moreover, an investigation of different parameters affecting the stability of produced nanoemulsion was conducted, in addition to the study of the effect of the nanoencapsulation of essential oils on their antibacterial activity. Finally, an enumeration of the advantages of encapsulating essential oils into nanoemulsion-based delivery systems in order to provide a natural food preservatives has been provided.

Keywords: encapsulation, nanoemulsion, essential oil, formulation, stability, antibacterial activity, food preservation

1. Introduction

In recent years, natural antimicrobials attracted consumer attention due to the increased awareness regarding food safety. In this context, new approaches have been adopted in the food preservation field. This includes the use of natural compounds with proven antibacterial activities, like essential oils, as safe preservatives. However, the incorporation of essential oils in foods is not economically and practically ideal. As a matter of fact, essential oils are not only volatile and chemically unstable in the presence of air, light, moisture and high temperatures, but also present hydrophobic properties.

With this respect, the nanoencapsulation of essential oils seems to be an attractive new approach to overcome these impediments.

Since the design of essential-oil-loaded particles is a complex process with interrelated steps [1], choosing encapsulating material and the encapsulation method should be in agreement with the intended matrix in which essential oils are to be introduced [2]. Basically, the nanoencapsulation of essential oils may imply coat, polymeric material, etc. to trap the core material in order to fix listed limitations of using essential oils as natural food preservative. Accordingly, different methods could be adopted for the nanoencapsulation of essential oils, such as nanoemulsion, liposomes, cyclodextrin, etc. In the specific case of essential oil nanoemulsion, the preparation consists on a biphasic liquid system of one liquid solution dispersed in a continuous medium and no polymer shells are used. The immobilization of essential oils in nanoemulsions contributes efficiently to enhance their dispersibility in aqueous solutions, to protect them from interaction with food ingredients, to minimize their impact on the organoleptic properties, as well as to improve their absorption and bioavailability. In this context, numerous researches have been conducted on the nanoencapsulation of essential oils on the purpose of producing a natural powerful food conservator. Gathered data demonstrated that inappropriate formulation, due to a misunderstanding of the process of essential oil encapsulation, can lead to the instability and/or the inefficiency of the produced emulsion.

In this context, the purpose of this chapter is to better understand the phenomenon of encapsulating essential oils into nanoemulsion-based delivery systems. This would widen the knowledge of possible alternatives to consider while designing green food preservatives for future research. Accordingly, this chapter covers firstly a general description of the nanoemulsion delivery systems. Then, an enumeration of common parameters, often used in essential oil nanoemulsion characterization, was conducted. The third part of this chapter involves different adopted methods for the preparation of essential oil nanoemulsion. Moreover, the most relevant parameters affecting the nanoemulsion quality and stability were investigated. Also a special emphasis to the effect of the nanoencapsulation of essential oils on their antibacterial activity was provided. Finally, data on the efficiency of encapsulated essential oils into nanoemulsion-based delivery systems as natural food preservatives have been provided.

2. Nanoemulsion-based delivery system as an example of bioactive compound encapsulation

According to the theory of emulsification, an emulsion is a thermodynamically unstable system consisting of at least two immiscible liquid phases, one of which is dispersed as globules in the other liquid phase [3]. Emulsions can be stabilized by increasing the repulsion between the dispersed and the continuous phases. As a matter of fact, the emulsion formation is a nonspontaneous phenomenon, which requires energy along with the use of emulsifiers. As a matter of fact, emulsifiers are amphiphile molecules that reduce the interfacial tension between the two phases and contribute to the stabilization of dispersed droplets with electrostatic or steric effects [4].

According to the proportion of each used liquid, an emulsion can be considered either as oil in water (O/W) or as water in oil (W/O) emulsion [2]. Indeed, if the oil droplets are dispersed throughout the aqueous phase, the emulsion is called oil-in-water (O/W). In the opposite case, where the water is dispersed as globules in the oil continuous phase, the emulsion is called water-in-oil emulsion (W/O).

It is worthy to mention the increasing interest accorded to multiple emulsions [1]. In this complex type of emulsion system, the W/O or O/W emulsions are dispersed in another immiscible liquid.

Accordingly, O/W/O emulsion is formed by very small oil droplets dispersed in water globules of a W/O emulsion, and W/O/W emulsion is formed by water droplets dispersed in the oil phase of an O/W emulsion (**Figure 1**). Multiple emulsion can be formed by a multistep mechanism. Actually, the deepest drop is formed in the first drop maker and then encapsulated in the next drop maker. In general, multiple emulsions present many advantages such as (1) a good ability to carry both hydrophilic and hydrophobic bioactive ingredients simultaneously, (2) high

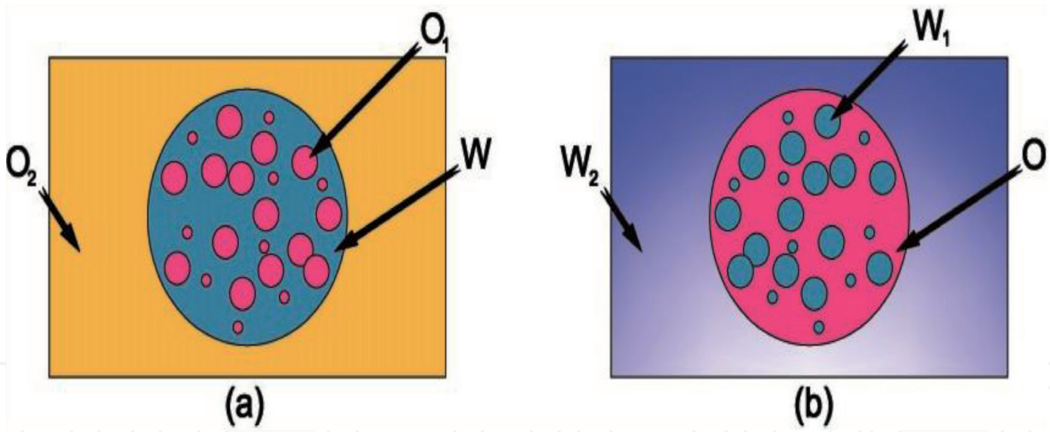

Figure 1.
Schematic representation of the architectures of O/W/O (a) and W/O/W (b) multiple emulsions.

protection of sensitive bioactive molecules from gastrointestinal harsh conditions, and (3) sheltering essential oil's strong taste and smell [1]. Besides their importance, multiple emulsions present some limitations due to their complex structure and their thermodynamic instability [5].

Another special case of emulsion is nanoemulsion. Actually, nanoemulsions are isotropic, clear, and kinetically stable with droplet size inferior to 200 nm [4]. Two types of techniques could be adopted for nanoemulsion preparation: high-energy methods and low-energy methods [6]. This encapsulation method is gaining more and more interest in the scientific community due to its high stability, as compared to emulsions of larger droplet size [7]. Actually, nanoemulsion stability results of its nanoscale droplet size and its large surface area and free energy. With this respect, essential oil nanoemulsion can be formed by the encapsulation of essential oil as the dispersed phase at a nanoscale level.

The main advantages of essential oil nanoemulsions are:

- it possess high kinetic stability;
- solubilize hydrophobic bioactive molecules and enhance their bioavailability;
- can be used for taste masking;
- nontoxic and nonirritant; and
- suitable for human and veterinary use.

In the last few decades, essential oil nanoemulsions have found enormous applications in the field of healthcare, cosmetics, food, agrochemicals, pharmaceuticals, and biotechnology.

2.1 Essential oil nanoemulsion components

The encapsulation of essential oils in nanoemulsion-based delivery system requires basically oil, emulsifier, and aqueous phase:

i. *Oils*: they are used to solubilize the lipophilic bioactive compound and to modulate the viscosity ratio between the dispersed and the continuous phases [8]. The commonly used oils in formulating essential oil food grade nanoemulsions are soyabean oil, ethyl oleate, sesame oil, castor oil, arachis oil, and corn oil.

ii. *Emulsifiers*: emulsifiers are amphiphilic molecules composed of two parts, polar and nonpolar regions [9]. According to their polar group nature, emulsifiers can also be classified into: anionic, cationic, nonionic, and zwitterionic emulsifiers. By lowering the interfacial tension between the two immiscible liquids, emulsifiers contribute significantly in the formulation of essential oil nanoemulsions [9]. Furthermore, they prevent coalescence of newly formed drops.

iii. *Aqueous phase*: the nanoemulsion stability is affected by the nature of the aqueous phase [4]. Particularly, consideration should be given to the aqueous phase pH and presence of electrolytes during nanoemulsion preparation. Theoretically, in essential oil nanoemulsion, continuous phase viscosity could influence droplet size through different mechanisms. It is worthy to mention that the relative importance of these mechanisms depends mainly on the homogenizer design and the used operating conditions [10].

2.2 Nanoemulsion characterization

As detailed in the literature, the majority of the researches, dealing with the essential oil encapsulation into nanoemulsion-based delivery system, have considered that the droplet size and distribution measurements as the most important parameters for their nanoemulsion characterization [4, 6, 11]. Measurements could be determined by laser light scattering, and obtained results are expressed in terms of mean particle size, which is usually represented [11] with Sauter mean diameter, $d_{3,2}$ (expressed in nm), calculated using the following equation:

$$d_{3,2} = (\text{Volume/Surface Area}) = (\sum (n_i * d_i)^3)/(\sum (n_i * d_i)^2) \quad (1)$$

where n_i is the number of droplets and d_i is the droplet diameter.

In addition to these two listed parameters, the stability of essential oil nanoemulsion, which means its ability to resist physicotemporal changes, could be investigated by storing nanoemulsions at different temperatures and periods and measuring at each time point the droplet size variation [12]. Also, some researches have measured the viscosity, the density, the color, the turbidity as physical characterization of their nanoemulsion [13, 14], while others have focused their researches on the investigation of the biological activities of produced nanoemulsions [11, 15].

2.3 Methods of nanoemulsion preparation

The laborious step of formulation aims to produce stable nanoemulsion. Indeed, any emulsion system, if inappropriately formulated, may be subject to a variety of physicochemical phenomena, which can seriously affect the stability and the biological efficiency of the produced nanoemulsion [16].

A well-homogenized and stable emulsion whose droplet diameters figure in the nanoscale level can only be obtained under a complex alliance between physical and chemical forces [17]. As a matter of fact, the exclusive use of physical forces remains often insufficient to obtain stable nanoemulsions. The aim role of physical forces is to reduce the size of the dispersed phase droplets at a certain level, while chemical interactions between different medium components interfere to maintain newly formed droplets from fusing together.

2.3.1 Chemical forces

From a chemical point of view, the interfacial tension remains a critical parameter in the process of essential oil nanoemulsification. As a matter of fact, interfacial tension is known as the inward attraction of molecules at the surface of immiscible liquids due to the imbalance of their attractive forces [18]. With this respect, the nanoemulsion formation can occur only if the interfacial tension between the two immiscible phases decreased sufficiently to assure their mixture [11]. To reach such change in interfacial tension, an appropriate amount of an appropriate emulsifier should be used to surround and stabilize all neo-formed nanodroplets. Indeed, an emulsifier is not only able to reduce the interfacial tension of the two immiscible phases, but also it presents an effective stabilizer for the newly formed droplets [19]. In this way, the surfactants convert large globules into small ones and avoid small globules from coalescing into large ones, by reducing the repellent force between the liquids and withdrawing the attraction of liquids for their own molecules [20]. It is worthy to mention that to ensure its fundamental role in the nanoemulsification process, surfactants should be used at a higher concentration than its critical micellar concentration "CMC." As a matter of fact, the increase of surfactant concentrations on oil-water interface increases the adsorption of surfactant molecules, leading to the improvement of their ability to reduce the interfacial tension. Once the adsorption saturation is reached at the oil-water interface, the adsorption of surfactant molecules would stop increasing; thus, the interfacial tension remains constant [21].

2.3.2 Physical forces

Physical emulsification is one of the most crucial steps in nanoencapsulation process since it affects deeply the quality of the final emulsion (encapsulation efficiency, nanoemulsion stability, or biological efficacy). Different homogenization methods, as detailed in **Table 1**, can be used such as high-pressure homogenization, ultrasonic homogenization, and microfluidization [22].

Devices	Encapsulated bioactive compound	Obtained particle size (nm)	Disadvantages	References
High-pressure homogenization	*Thymus capitatus* essential oil	110	- High production costs	[11]
	Melaleuca alternifolia essential oil	175		[2]
Microfluidization	*Eugenia caryophyllata* essential oil	21	- High production costs - Less aseptic processing	[23]
	Lemon myrtle essential oil	97		[15]
Sonication	Rosemary essential oil	187	- Heat generation during the process due to high shear forces and cavitations	[24]
	Thymus vulgaris essential oil	121		[25]

Table 1.
The different devices often used for the nanoencapsulation of essential oils.

High-pressure homogenization: the use of high-pressure (30–350 MPa) homogenization can reduce an emulsion droplet size to a nanoscale level and improve its stability by the reduction of the creaming rate. In this method, two immiscible liquids along with the used emulsifier are forced to pass through a small orifice where their mixture is subjected to a turbulence and shear flow intense levels [2]. All of which leads to the break-up of the dispersed phase into small droplets.

Microfluidization: this technic involves the transfer of mechanical energy to fluid particles under a high-pressure environment. More specifically, immiscible liquids are pumped and split into two opposite microstreams, which are impacted or collided against each other in a chamber, called the interaction chamber, where shear, turbulent and cavitation forces are generated using high-pressure displacement pump [9].

Ultrasonication: the droplet formation at a nanoscale level using ultrasound homogenization mechanisms is mainly based on cavitation, where ultrasound waves hit the liquid surface and form high-velocity jets. To do that, a probe sonicator is brought in contact with the dispersion of emulsifier and liquids. The generated mechanical vibration and cavitation can provide the necessary energy input for the formation of small sized droplets [26].

2.4 Parameters affecting nanoemulsion droplet size and stability

The droplet homogeneity, and consequently emulsion stability, depends basically on the physical characteristics of each used component for the formation of essential oil nanoemulsion [9]. **Table 2** summarizes the obtained droplet size of different tested formulation:

2.4.1 Dispersed phase characteristics

During the process of encapsulating essential oils into nanoemulsion-based delivery system, dispersed phase characteristics influence profoundly the final product properties [3]. In this context, the physical characteristics of the encapsulated essential oil (such as interfacial tension and viscosity) present a key factor in the nanoemulsion stability [11]. For example, it is difficult to nanoencapsulate pure fixed oils due to their high viscosity. Indeed, if the dispersed phase viscosity increases, it will become more difficult to breakup within the high-pressure homogenizer. As a result, nanoemulsions with larger droplets will be formed. This phenomenon was also confirmed by other researches for various types of homogenization device, who declared that the droplet breakup becomes easier as the viscosity of the dispersed phase decreases [30]. Also, the increase of the dispersed phase viscosity generated a significant increase of the mean droplet diameter, from around 92 to around 125 nm [9]. Other researches demonstrated that instantaneous unstable emulsions were obtained while trying the nanoencapsulation of pure essential oil [11]. These

Encapsulated essential oil	Dispersed phase	Emulsifier	Droplet size (nm)	References
Thymus capitatus	Mixture with 30% of soybean oil	SDS	110	[11]
Eucalyptus globulus	Pure essential oil	Tween 20	60	[27]
Carvacrol	Mixture with 60% of medium chain triglyceride	Tween 80	100	[28]
Peppermint	Pure essential oil	Tween 80	70	[29]

Table 2.
Formulation effect on the droplet size of essential oil nanoemulsion.

findings were explained by the lower viscosity of pure essential oils. Actually, to obtain a stable emulsion with a nanoscale droplet size, there is an optimum range of disperse-to-continuous phase viscosity ratios [10]. With this respect, it would be convenient to nanoencapsulate a mixture of essential oil and fixed oil as dispersed phase in order to obtain an essential oil nanoemulsion.

2.4.2 Continuous phase characteristics

Changing the continuous phase viscosity influences the nanoemulsion droplet size [9]. More specifically, the increase of the continuous phase viscosity leads to the droplet diameter decrease [10]. Actually, the increase of the continuous phase viscosity induces the increase of the disruptive shear stresses, which leads to the increase of droplet fragmentation.

2.4.3 Emulsifier chemical characteristics

Although a wide range of molecules may be used in the essential oil nanoemulsification (exp. colloids, special particles), only the emulsifier case will be presented in this chapter. Different systems were adopted in order to classify surfactants. For instance, a very useful system for the classification of surfactants is standardized on the basis of their solubility in water. In this system, numerical values are called the hydrophilic-lipophilic balance (HLB), which involves the relative affinity of the surfactant for water and oil. The HLB is defined as the relative efficiency of the hydrophilic portion of the surfactant molecule to its lipophilic portion. It is worthy to mention that emulsifiers with HLB values ranged between 3 and 6 are usually used for w/o emulsions. Whereas emulsifiers with HLB values ranged between 7 and 20 are used for o/w emulsions [5]. Besides, other researchers have based their investigations on the effect of emulsifier chemical nature in producing homogenous nanoemulsions [9, 11, 31]. Their findings confirmed that emulsifier type presents significant impact on the final emulsion stability. This variation of emulsifier behaviors could be explained by the difference in the stabilization process of each one. Previous researches demonstrated that under similar homogenization conditions, small-molecule emulsifiers (exp. Tween and SDS) can be more effective to make small droplets than biopolymers (exp. caseinate and β-lactoglobulin) due to their rapid adsorption to the droplet surfaces [9]. Moreover, charged emulsifiers can be more efficient in producing homogenous nanoemulsions as compared to nonionic ones [11]. In fact, contrary to nonionic emulsifier, which uses steric repulsion to stabilize the dispersed phase, charged emulsifiers use their electrostatic repulsion. Actually, for nonionic emulsifier-based emulsion, tails envelop essential oil inside the droplet [32, 33]. In this case, the hydrophilic nature of tails may repel the hydrophobic essential oils leading to a significant heterogeneity of the droplet diameters. In anionic emulsifier-based emulsions, the inverse occurs. As a matter of fact, the adsorption of negatively charged heads of emulsifier molecules to oil droplets surface increases the electrostatic repulsion between droplets, leading to the formation of a stable nanoemulsion [34]. In this case, the charged heads of SDS molecules envelop essential oil inside the droplet, while the hydrophilic tails stay outside leading to an appropriate homogeneous nanoemulsion.

2.5 Nanoemulsion instability

Nanoemulsions lose their stability, which is an irreversible phenomenon in nature, in a very large time frame that may vary from few minutes to several years, depending on formulation and storage conditions [35]. In this context, nanoemulsions stability can be checked according to their droplet size growth and their appearance.

Generally, emulsion stability depends mainly on emulsifier behavior, emulsion composition, and its droplet size distribution [3]. Indeed, nanoemulsions, due to their characteristic nanoscale droplets size, exhibit higher stability against creaming or sedimentation, than emulsions with larger droplet diameters. Actually, diffusion rate and Brownian motion exhibited by nanoemulsion droplets predominates over the sedimentation or the creaming rate [4].

Concerning the emulsifier behavior, nanoemulsions prepared using nonionic surfactants do not usually flocculate, as no attractive forces are created [11].

Also, nanoemulsion stability depends strongly on their storage time and conditions. Actually, small droplets of freshly made nanoemulsion could initially be distributed in the medium, but are rather unstable, resulting in droplet growth during long storage, and these new large droplets are the source of flocks. In fact, droplets flocculation appears whenever the interfacial tension of the dispersed phase is weaker than its own net attractive forces [36]. Accordingly, nanoemulsions storage temperature increase not only provokes the increase in molecules thermal agitation [37], but also the decrease in their interfacial tension. Consequently, the droplet diameter of instable thermodynamic nanoemulsions would tend to increase to reduce medium total free energy. The nanoemulsion storage at high temperature (up to 55°C) generated new populations of larger droplets after 15 storage days [9].

Worthy to note that nanoemulsion instability occurs due to alteration in droplet size through mechanisms such as Coalescence and Ostwald ripening.

i. *Coalescence:* this phenomenon results from the fusion of two or more droplets into one larger one by the breakdown of the thin film existing between them [38]. As a matter of fact, coalescence occurs if the adhesion force between two droplets exceeds the turbulent force that creates the dispersion. Coalescence can be prevented by the addition of emulsifiers, which have the same charges causing repulsion between two droplets [4].

ii. *Ostwald ripening:* this phenomenon is characterized by change in droplet size and distribution, as well as by turbidity apparition in nanoemulsions [3]. Actually, Ostwald ripening occurs with time passage due to the migration of droplets from the dispersed phase (high Laplace pressure) to the continuous phase (low Laplace pressure), leading to molecular diffusion [39]. To prevent Ostwald ripening, several parameters should be taken in consideration such as: the physical properties of the bioactive compound, the mutual solubility of the phases, the nature and concentration of used emulsifier, preparation methods, and storage conditions [4].

Otherwise, practically nanoemulsions are usually stored at lower temperatures, inducing therefor, their longer stability and higher resistance to droplet aggregation.

2.6 Effect of the nanoencapsulation process on essential oils antibacterial efficiencies

As the antibacterial efficiency is a fundamental characteristic of essential oils, different methods were adopted in order to seek the effect of the nanoencapsulation process on essential oil antibacterial potency [11, 25, 40]. All results depicted clear amelioration in the antibacterial efficiency. Such amelioration suggests a refinement of the mode of action of essential oils after their nanoencapsulation in fighting pathogenic bacteria. Actually, some researchers considered the nanoemulsion as a transporter for essential oils to cross the bacterial cellular membrane, allowing them to overcome their hydrophobic limitation [11]. Indeed, essential oils exert their known antibacterial effect from the inner side of the cytoplasmic membrane [28]. Essential oil antibacterial effect is based on their abilities to disrupt the bacterial cytoplasmic membrane to lose its properties as a barrier, matrix for enzymes, and energy transducer, all of which will compromise the cell viability leading to its death [41, 42]. However, essential oil presents low water solubility, inducing its rough distribution in the medium, which can limit its antibacterial action [28, 42].

Moreover, it is worthy to note that the interesting antibacterial activity of nanoencapsulated essential oil could present a promising procedure to fight against the global issue involving drug-resistant strains. As a matter of fact, in addition to the antibacterial activity amelioration of essential oil after their nanoencapsulation, many essential oils have succeeded to surpass the efficiency of current antibiotic. These findings are very promoting, especially that the development of drug-resistant strains has become a worldwide concern [43]. As a matter of fact, typical antibiotic killing technique consists on blocking bacterial ribosome formation at the initiation step [44]. Consequently, no protein synthesis, which is obligatory for bacterial metabolism and survival, takes place leading the bacterial cell death. In this context, antibiotic-resistant strain could be formed after mutation of the initial bacteria whose ribosome formation continues even in the presence of the drug. However, the efficiency of nanoencapsulated essential oil, based on the nonspecific disruption of bacterial cell membranes, can resist bacterial mutation and maintain its bactericidal activities. Accordingly, the use of nanoencapsulated essential oils into nanoemulsion-based delivery system is very efficient to fight pathogenic bacteria and would not conduct the development of resistant strains, which could remediate to the bacterial resistance problem, caused by the widespread and inappropriate use of antibiotics [11].

2.7 Nanoencapsulated essential oils as efficient food conservators

It has been repeatedly demonstrated that essential oil efficiency can be reduced in real food systems due to their hydrophobic character and their low solubility in water as compared to *in vitro* model system [11, 45]. This reduce in essential oil activity is more noticed in foods with high fat level such as milk, mayonnaise, butter, etc. [46]. For instance, it has been demonstrated that in cheese, an increase up to 100-fold of the essential oil concentration was required to assure comparable antimicrobial efficacy of the *in vitro* model system [47]. The difference in the essential oil antimicrobial efficiency between *in vitro* and real food system can be attributed to the essential oil dissolution in the lipid phase of an ailment, inducing the decrease of their concentration in the aqueous phase [48]. However, it is in the aqueous phase where pathogenic bacteria typically proliferate [46]. Consequently, to ensure similar antimicrobial activity in *in vitro* and in real food system, essential oils have to be relocated in the aqueous phase of the food, in order to be in continuous contact with the pathogenic microorganisms. In order to accomplish this goal, the encapsulation of essential oils into a nanoemulsion-based delivery system seems to be an interesting approach. As a matter of fact, the hydrophilic outer surface of the nanoemulsion enables essential oils to stay in the food's aqueous phase, while its hydrophobic inner core ensures its harbor.

Also, the nanoencapsulation of essential oils prevents their interaction with food components, which induce a positive impact on essential oil antimicrobial efficiency. Indeed, bulk essential oils tend to bind with hydrophobic food molecules leading to the reduction of their availability to fight pathogenic microorganisms [48]. In contrast, nanodispersed essential oil is evenly distributed in food matrix and can be released locally to keep its concentration sufficiently high to inhibit the growth of the spoilage bacteria [49].

Moreover, designing systems that entrap essential oil molecules can reduce the adverse interaction of their characteristic aroma with the original food flavor. As a matter of fact, the use of essential oils as food conservatives can be limited by their sensorial impact on the final food product. Accordingly, adding *Lavandula* and *Chamaemelum* spp. essential oils to yoghurt decreased its acceptability by panelist [50]. Actually, when incorporated into food system, bulk essential oils bind with fats [51]. Such bindings could alter the sensory appreciation of the incorporated aliment, since the taste appreciation depends mainly on its fat quality [52]. On the other way, the encapsulation of *Thymus capitatus* essential oil, into a nanoemulsion-based delivery system, ameliorated significantly its sensorial impact when incorporated into milk [48]. Authors explained their findings by the fact that, when nanoencapsulated, essential oil components were trapped inside droplets and were not able to interact with milk ingredients [53]; therefore, their incorporation would not modify fat quality.

3. Conclusion

The encapsulation of natural bioactive compounds into a nanoemulsion-based delivery system presents definitely an interesting approach to facilitate and ameliorate the valorization of essential oils as natural and green food conservators. Actually, the nanoencapsulation of essential oils protect them from brutal external conditions, ameliorate their distribution in the medium leading to an amelioration of their bactericidal potency, as well as prevent their interaction with food components, which induce a positive impact on their incorporation efficacy. However, special attention should be attributed to the formulation step of essential oil nanoemulsion to avoid different physicochemical phenomena, which can seriously affect the stability and the biological efficiency of the produced nanoemulsion.

Acknowledgements

This study was supported by the Tunisian Ministry of Higher Education, Scientific Research, Information, and Communication Technologies.

Conflict of interest statement

The authors declare that there are no conflict of interests.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author details

Mariem Ben Jemaa*, Hanen Falleh and Riadh Ksouri
Laboratory of Aromatic and Medicinal Plants, Biotechnology Center of Borj Cedria, Hammam-Lif, Tunisia

*Address all correspondence to: mariembenjemaa@yahoo.fr

References

[1] Sagiri SS, Anis A, Pal K. A review on encapsulation of vegetable oils: Strategies, preparation methods and applications. Polymer-Plastics Technology and Engineering. 2015;**55**(3):291-311. DOI: 10.1080/03602559.2015.1050521

[2] Donsi F, Annunziata M, Sessa M, Ferrari G. Nanoencapsulation of essential oils to enhance their antimicrobial activity in foods. LWT—Food Science Technology. 2011;**44**(9):1908-1914. DOI: 10.1016/j.lwt.2011.03.003

[3] Tadros T, Izquierdo P, Esquena J, Solans C. Formation and stability of nanoemulsions. Advance in Colloid and Interface Science. 2004;**108**:303-318. DOI: 10.1016/j.cis.2003.10.023

[4] Setya S, Talegaonkar S, Razdan BK. Nanoemulsions: Formulation methods and stability aspects. World Journal of Pharmacy And Pharmaceutical Sciences. 2014;**3**(2):2214-2228

[5] Khan BA, Akhtar N, Khan HMS, Waseem K, Mahmood T, Rasul A, et al. Basics of pharmaceutical emulsions: A review. African Journal of Pharmacy and Pharmacology. 2011;**5**(25): 2715-2725. DOI: 10.5897/AJPP11.698

[6] Gupta A, Burak Eral H, Alan Hattona T, Doyle PS. Nanoemulsions: Formation, properties and applications. The Royal Society of Chemistry. 2016;**12**:2826-2841 DOI: 10.1039/c5sm02958a

[7] Qadir A, Faiyazuddin MD, Talib Hussain MD, Alshammari TM, Shakeel F. Critical steps and energetics involved in a successful development of a stable nanoemulsion. Journal of Molecular Liquids. 2016;**214**:7-18. DOI: 10.1016/j.molliq.2015.11.050

[8] Zhang Y, Gao J, Zheng H, Zhang R, Han Y. The preparation of 3,5-dihydroxy-4-isopropylstilbene nanoemulsion and in vitro release. International Journal of Nanomedecine. 2011;**6**:649-657. DOI: 10.2147/IJN.S17242

[9] Qian C, McClements DJ. Formation of nanoemulsions stabilized by model food-grade emulsifiers using high-pressure homogenization: Factors affecting particle size. Food Hydrocolloids. 2011;**25**:1000-1008. DOI: 10.1016/j.foodhyd.2010.09.017

[10] Håkansson A, Tragårdh C, Bergenståhl B. Studying the effects of adsorption, recoalescence and fragmentation in a high-pressure homogenizer using a dynamic simulation model. Food Hydrocolloids. 2009;**23**(4):1177-1183. DOI: 10.1016/j.foodhyd.2008.10.003

[11] Ben Jemaa M, Neves MA, Falleh H, Isoda H, Ksouri R, Nakajima M. Nanoencapsulation of *Thymus capitatus* essential oil: Formulation process, physical stability characterization and antibacterial efficiency monitoring. Industrial Crops and Products. 2018;**113**:414-421. DOI: 10.1016/j.indcrop.2018.01.062

[12] Rodríguez-Rojo S, Visentin A, Maestri D, Cocero MJ. Assisted extraction of rosemary antioxidants with green solvents. Journal of Food Engineering. 2012;**109**(1):98-103. DOI: 10.1016/j.jfoodeng.2011.09.029

[13] Basak S, Guha P. Betel leaf (*Piper betle* L.) essential oil microemulsion: Characterization and antifungal activity on growth, and apparent lag time of *Aspergillus flavus* in tomato paste. LWT–Food Science and Technology. 2017;**75**:616-623. DOI: 10.1016/j.lwt.2016.10.021

[14] Ghosh V, Mukherjee A, Chandrasekaran N. Formulation and

characterization of plant essential oil based nanoemulsion:Evaluation of its larvicidal activity against *Aedes aegypti*. Asian Journal of Chemistry. 2013;**25**:321-323

[15] Buranasuksombat U, Kwon YJ, Turner M, Bhandari B. Influence of Emulsion Droplet Size on Antimicrobial Properties. Food Science and Biotechnology. 2011;**20**(3):793-800. DOI: 10.1007/s10068-011-0110-x

[16] Chang Y, McLandsborough L, McClements DJ. Physical properties and antimicrobial efficacy of thyme oil nanoemulsions: Influence of ripening inhibitors. Journal of Agricultural and Food Chemistry. 2012;**60**:12056-12063. DOI: 10.1021/jf304045a

[17] Wooster TJ, Golding M, Sanguansri P. Impact of oil type on nanoemulsion formation and Ostwald ripening stability. Langmuir. 2008;**24**(22): 12758-12765. DOI: 10.1021/la801685v

[18] John EH. Dispensing of Medication. Pennysylvania, USA: Mack Publishing Company; 1976. pp. 345-358

[19] Walstra P. Principles of emulsion formation. Chemical Engineering Science. 1993;**48**:333-349. DOI: 10.1016/0009-2509(93)80021-H

[20] Paul BK, Mitra RK. Water solubilization capacity of mixed reverse micelles: Effect of surfactant component, the nature of the oil, and electrolyte concentration. Journal of Colloids and Interface Science. 2005;**288**:261-279. DOI: 10.1016/j.jcis.2005.02.088

[21] Yuan C, Pu WF, Wang XC, Sun L, Zhang Y, Cheng S. The effects of interfacial tension, emulsification and surfactant concentration on oil recovery in surfactant flooding process for high temperature and high salinity reservoirs. Energy and Fuels. 2016;**55**(48):12387-12397. DOI: 10.1021/acs.energyfuels.5b01393

[22] McClements DJ. Nanoemulsions versus microemulsions: Terminology, differences, and similarities. Soft Matter. 2012;**8**:1719-1729. DOI: 10.1039/C2SM06903B

[23] Salvia-Trujillo L, Rojas-Graü A, Soliva-Fortuny R, Martín-Belloso O. Physicochemical characterization and antimicrobial activity of foodgrade emulsions and nanoemulsions incorporating essential oils. Food Hydrocolloids.2014;**43**:547-556. DOI: 10.1016/j.foodhyd.2014.07.012

[24] Koh LL, Chandrapala J, Zisu B, Martin GJ, Kentish SE, Ashokkumar M. A comparison of the effectiveness of sonication, high shear mixing and homogenisation on improving the heat stability of whey protein solutions. Food Bioprocess Tehnology. 2014;**7**:556-566. DOI: 10.1007/s11947-013-1072-1

[25] Ghaderi-Ghahfarokhi M, Barzegar M, Sahari MA, Azizi MH. Nanoencapsulation approach to improve antimicrobial and antioxidant activity of thyme essential oil in beef burgers during refrigerated storage. Food Bioprocess Technology. 2016;**9**(7):1187-1201. DOI: 10.1007/s11947-016-1708-z

[26] Delmas T, Piraux H, Couffin AC, Texier I, Vinet F, Poulin P, et al. How to prepare and stabilize very small nanoemulsions. Langmuir. 2010;**27**(5):1683-1692. DOI: 10.1021/la104221q

[27] Sugumar S, Mukherjee A, Chandrasekaran N. Nanoemulsion formation and characterization by spontaneous emulsification: Investigation of its antibacterial effects on *Listeria monocytogenes*. Asian Journal of Pharmaceutics. 2015;**9**:23-28. DOI: 10.22377/ajp.v9i1.427

[28] Landry KS, Chang Y, McClements DJ, McLandsborough L. Effectiveness of a novel spontaneous carvacrol nanoemulsion against *Salmonella enterica Enteritidis* and *Escherichia coli* O157:H7 on contaminated mung bean and alfalfa seeds. International Journal of Food Microbiology. 2014;**187**:15-21. DOI: 10.1016/j.ijfoodmicro.2014.06.030

[29] Barzegar H, Mehrnia MA, Nasehi B, Alipour M. Fabrication of peppermint essential oil nanoemulsions by spontaneous method: Effect of preparing conditions on droplet size. Flavor and fragrance Journal. 2018;**33**(5):351-356. DOI: 10.1002/ffj.3455

[30] Jafari SM, Assadpoor E, Bhandari B, He Y. Nano particle encapsulation of fish oil by spray drying. Food Research International. 2008;**41**(2):172-183. DOI: 10.1016/j.foodres.2007.11.002

[31] Reynhout XEE, Hoekstra L, Meuldijk J, Drinkenburg AAH. Contribution of steric and electrostatic repulsion forces to the stability of styrene latices copolymerized with acrylic acid. Steric and Electrostatic Repulsion Forces. 2003;**41**(19):2985-2995. DOI: 10.1002/pola.10889

[32] Nash JJ, Erk KA. Stability and interfacial viscoelasticity of oil-water nanoemulsions stabilized by soy lecithin and tween 20 for the encapsulation of bioactive carvacrol. Colloids and Surfaces A: Physicochemistry and Engenieering Aspects. 2017;**517**:1-11. DOI: 10.1016/j.colsurfa.2016.12.056

[33] Li X, Li H, Xiao Q, Wang L, Wang M, Lu X, et al. Two-way effects of surfactants on Pickering emulsions stabilized by the self-assembled microcrystals of α-cyclodextrin and oil. Physical Chemistry Chemical Physics. 2014;**16**:14059-14069. DOI: 10.1039/c4cp00807c

[34] Krstonosic V, Dokic L, Nikolic I, Dapcevc T, Hadnadev M. Influence of sodium dodecyl sulphate (SDS) concentrations on the disperse and rheological characteristic of oil in water emulsion stabilized by octenyl succinic anhydride modified starch-SDS mixture. Journal of Sebian Chemistry Society. 2012;**77**:83-94. DOI: 10.2298/JSC100630150K

[35] Reza A. Nanometric-scaled emulsions (nanoemulsions). Iranian Journal of Pharmaceutic Research. 2010;**9**(4):325-326

[36] Kim HJ, Decker EA, McClements DJ. Impact of protein surface denaturation on droplet flocculation in hexadecane oil-in-water emulsions stabilized by beta-lactoglobulin. Journal of Agricultural and Food Chemistry. 2002;**50**(24):7131-7137. DOI: 10.1021/jf020366q

[37] Taylor L. The Healing Power of Rainforest Herbs. A Guide to Understanding and Using Herbal Medicinals. New York: Square One Publishers; 2005

[38] Delmas T, Piraux H, Couffin AC, Texier I, Vinet F, Poulin P, et al. How to prepare and stabilize very small nanoemulsions. Langmuir. 2010;**27**(5):1683-1692

[39] Tadros TF. Emulsion science and technology: A general introduction. In: Tadros TF, editor. Emulsion Science and Technology. Weinheim: Wiley-VCH; 2009. DOI: 10.1002/9783527626564.ch1

[40] Bilia AR, Santomauro F, Sacco C, Bergonzi MC, Donato R. Essential oil of *Artemisia annua* L.: An extraordinary component with numerous antimicrobial properties. Trends Plant Science. 2014;**9**:97-104. DOI: 10.1155/2014/159819

[41] Turina AV, Nolan MV, Zygadlo JA, Perillo MA. Natural terpenes: Self-assembly and membrane partitioning.

Biophysical Chemistry. 2006;**122**: 101-113. DOI: 10.1016/j.bpc.2006.02.007

[42] Gill AO, Holley RA. Disruption of *Escherichia coli*, *Listeria monocytogenes* and *Lactobacillus sakei* cellular membranes by plant oil aromatics. International Journal of Food Microbiology. 2006;**108**:1-9. DOI: 10.1016/j.ijfoodmicro.2005.10.009

[43] Zaman SB, Hussain MA, Nye R, Mehta V, Mamun KT, Hossain N. A review on antibiotic resistance: Alarm bells are ringing. Cureus. 2017;**9**(6):1403-1411. DOI: 10.7759/cureus.1403

[44] Luzzatto L, Apirion TD. Schlessinger D, Mechanism of action of Streptomycin in *E. coli*: Interruption of the ribosome cycle at the initiation of protein synthesis. Proceedings of the National Academy of Sciences. 1968;**60**:873-880

[45] Bonnaud M, Weiss J, McClements DJ. Interaction of a food-grade cationic surfactant (lauric arginate) with food-grade biopolymers (pectin, carrageenan, xanthan, alginate, dextran, and chitosan). Journal of Agricultural and Food Chemistry. 2010;**58**:9770-9777. DOI: 10.1021/jf101309h

[46] Edris AE. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: A review. Phytotherapy Research. 2007;**21**:308-323. DOI: 10.1002/ptr.2072

[47] Mendoza-Yepes MJ, Sanchez-Hidalgo LE, Maertens G, Marinlniesta F. Inhibition of *Listeria monocytogenes* and other bacteria by a plant essential oil (DMC) in Spanish soft cheese. Journal of Food Safety. 1997;**17**:47-55. DOI: 10.1111/j.1745-4565.1997.tb00175.x

[48] Ben Jemaa M, Falleh H, Neves MA, Isoda H, Nakajima M, Ksouri R. Quality preservation of deliberately contaminated milk using thyme free and nanoemulsified essential oils. Food Chemistry. 2017:726-734. DOI: 10.1016/j.foodchem.2016.09.030

[49] Shah B, Davidson PM, Zhong Q. Nanodispersed eugenol has improved antimicrobial activity against *Escherichia coli* O157:H7 and *Listeria monocytogenes* in bovine milk. International Journal of Food Microbiology. 2013, 2013;**161**: 53-59. DOI: 10.1016/j.ijfoodmicro

[50] Ghalem BR, Zouaoui B. Evaluation of the quality of steamed yogurt treated by *Lavandula* and *Chamaemelum* species essential oils. Journal of Medicinal Plants Research. 2013;**7**(42):3121-3126. DOI: 10.5897/JMPR12.1037

[51] Shah GM, Groot JCJ, Oenema O, Lantinga EA. Covered storage reduce losses and improve crop utilisation of nitrogen from solid cattle manure. Nutrient Cycling in Agroecosystems. 2012;**94**:299-312. DOI: 10.1007/s10705-012-9543-8

[52] Guinard JX, Mazzucchelli R. Effects of sugar and fat on the sensory properties of milk chocolate: Descriptive analysis and instrumental measurements. Journal of the Science of Food and Agriculture. 1999;**79**: 1331-1339. DOI: 10.1002/(SICI)1097-0010(199908)79:11<1331::AID-JSFA365>3.0.CO;2-4

[53] Selim S. Antimicrobial activity of essential oils against vancomycin-resistant *Enterococci* (vre) and *Escherichia coli* o157:h7 in feta soft cheese and minced beef meat. Brazilian Journal of Microbiology. 2011;**42**:187-196. DOI: 10.1590/S1517-83822011000100023