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Physicochemical characterization and antimicrobial activity of food-grade emulsions and nanoemulsions incorporating essential oils

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ABSTRACT

Coarse emulsions containing essential oils (lemongrass, clove, tea tree, thyme, geranium, marjoram, palmarosa, rosewood, sage or mint) and stabilized with tween 80 and sodium alginate were prepared by high shear homogenization. Nanoemulsions were obtained by microfluidization of coarse emulsions. In general, the average droplet size of coarse emulsions was dramatically reduced after microfluidization down to a few nanometers, with the exception of palmarosa and rosewood oil emulsions, which were already in the nano-range before being treated. The ζ-potential of nanoemulsions exhibited values more negative than −30 mV, indicating a strong electrostatic repulsion of the dispersed oil droplets in the aqueous phase. The viscosity of nanoemulsions significantly decreased after microfluidization, with at least a 30% drop in their initial values. The whiteness index of nanoemulsions diminished after being treated. In fact, nanoemulsions containing tea tree, geranium or marjoram essential oils became completely transparent after microfluidization. Lemongrass, clove, thyme or palmarosa-loaded nanoemulsions were those with a higher *in vitro* bactericidal action against *Escherichia coli*, as they achieved 4.1, 3.6, 2.8 or 3.9 log-reductions after 30 min of contact time. In addition, a faster and enhanced inactivation kinetic was observed in the case of nanoemulsions containing lemongrass or clove essential oils in comparison with their respective coarse emulsions. Thus, the present work evidences the promising advantages of using nanoemulsions as delivery systems of flavoring and preservative agents in the food industry.

1. Introduction

Essential oils contain a complex mixture of non-volatile and volatile compounds produced by aromatic plants as secondary metabolites (Bakkali, Averbeck, Averbeck, & Idaomar, 2008; Burt, 2004). They are used in a wide variety of applications in food, pharmaceutical and cosmetics industries due to their flavoring, antioxidant and antimicrobial properties (Adorjan & Buchbauer, 2010; Brud, 2010; Tajkarimi, Ibrahim, & Cliver, 2010). In particular, the antimicrobial action of essential oils has been attributed to their phenolic compounds and their interaction with microbial cell membranes. They are known to penetrate through the microbial membrane and cause the leakage of ions and cytoplasmatic content thus leading to cellular breakdown (Burt, 2004). The interest of incorporating essential oils in foods as preservatives is related to their recognition as safe natural compounds, being a potential alternative to produce foods free of synthetic additives. However, the incorporation of antimicrobial essential oils to foods still presents several drawbacks due to their poor water solubility as well as to toxicological and economical considerations (Burt, 2004; Sánchez-González, Vargas, González-Martínez, Chiralt, & Cháfer, 2011). In addition, their strong flavor makes incorporation at high doses difficult in certain types of food products due to possible objectionable sensory characteristics. Hence, there is a need to investigate new delivery systems to encapsulate and release essential oils in food products.

In the food sector, the incursion of nanotechnological advances is still discreet but it is gaining more and more interest by both the scientific and industrial community (Rashidi & Khosravi-Darani, 2011). Recently, emulsions with small droplet size, typically from 10 to 100 nm, also called nanoemulsions, are being investigated as lipophilic drug delivery systems in food, cosmetic and pharmaceutical products (Bernardi et al., 2011; He et al., 2011). Due to their intrinsic properties, they may present several advantages for encapsulating functional lipophilic compounds over conventional emulsions. On the one hand, their reduced droplet size might not only enhance the transport of active molecules through biological

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membranes but also increase the surface area/volume ratio, thus leading to improved functionality. On the other hand, nanoemulsions are kinetically stable and transparent colloidal dispersions, being suitable for a wide range of practical applications (Solans, Izquierdo, Nolla, Azemar, & Garcia-Celma, 2005).

To produce nanoemulsions with droplet sizes in the nano-range a high shear rate is required in order to generate a high interface area (Delmas et al., 2011). Among the few methods that can produce nanoemulsions, microfluidization has been shown to generate extremely fine emulsions with droplet sizes between 60 and 600 nm (Hatanaka et al., 2010; Hatanaka, Kimura, Lai-Fu, Onoue, & Yamada, 2008; Jafari, He, & Bhandari, 2007a, 2007b; Qian & McClements, 2011; Rao & McClements, 2011; Wooster, Golding, & Sanguansri, 2008). The microfluidizer device uses a pump to force a coarse emulsion pre-mix to an interaction chamber under high pressure (Jafari et al., 2007a; McClements, 2011). In the interaction chamber the coarse emulsion is split in two streams that further impinge on each other at high velocity, providing an exceptionally fine emulsion (Mahdi Jafari, He, & Bhandari, 2006; McClements, 2011).

The use of nanoemulsions as potential delivery systems of lipophilic food ingredients is arising with promising expectations. However, there is a lack of scientific evidence about the enhanced functionality of nanoemulsions in comparison with similar non-nano-formulated delivery systems (Bouwmeester et al., 2009). Therefore, the purpose of the present research work was to characterize microfluidized nanoemulsions incorporating essential oils in terms of droplet size, size distribution, ζ-potential, viscosity and color in comparison with conventional emulsions. Moreover, the *in vitro* antimicrobial activity against *Escherichia coli* of nanoemulsions was assessed and compared with that exhibited by conventional emulsions.

2. Material and methods

2.1. Primary emulsion formation

Sodium alginate (FMC Biopolymers, UK) was dissolved in hot water at 70 °C (1% w/v) under continuous stirring until complete homogenization. Coarse emulsions were prepared by mixing the resulting sodium alginate aqueous solution with essential oils (1% v/v). Sodium alginate was included in the aqueous phase as the purpose of further investigations was to apply such nanoemulsions as antimicrobial edible coatings in fresh-cut fruit. Lemongrass (*Cymbopogon citratus*) essential oil was purchased at Laboratoris Dicana (Spain) and clove (*Eugenia caryophyllata*), tea tree (*Melaleuca alternifolia*), thyme (*Thymus vulgaris*), geranium (*Pelargonium graveolens*), marjoram (*Origanum majorana*), palmarosa (*Cymbopogon martinii*), rosewood (*Aniba rosaedora*), sage (*Salvia officinalis*) and mint (*Mentha spicata*) were purchased from Dietetica Intersa (Spain). The composition of each type of oil is detailed in Table 1, according to the information given by the supplier. Tween 80 (1% v/v) (Scharlau, Spain) was added as surfactant with a laboratory T25 digital Ultra-Turrax mixer (IKA, Staufen, Germany) working at 3400 rpm for 2 min. All samples were prepared using ultra pure water obtained from a Mili-Q filtration system.

2.2. Nanoemulsion formation

A microfluidization system (M110P, Microfluidics, Massachusetts, USA) was used to obtain nanoemulsions from the coarse emulsions. Coarse emulsions were passed through the system 3 times at a constant pressure of 150 MPa. At the outlet of the interaction chamber the product was refrigerated through an external cooling coil immersed in an ice-water bath so that the temperature of the product was always kept below 20 °C.

2.3. Emulsion and nanoemulsion characterization

2.3.1. Particle size and ζ-potential

The oil droplet size was measured by dynamic light scattering (DLS) with a Zetasizer NanoZS laser diffractometer (Malvern Instruments Ltd, Worcestershire, UK) working at 633 nm at 25 °C and equipped with a backscatter detector (173°), which is appropriate to measure sub-micron particles (Brar & Verma, 2011). DLS measures the Brownian motion of nano-sized droplets and relates this movement to an equivalent hydrodynamic diameter (nm). Average droplet size, size distribution curves and polydispersity index were used to characterize oil droplets dispersion in nanoemulsions. The refractive index of essential oils and their absorbance at 633 nm, were measured with a refractometer and a spectrophotometer, respectively (Table 1).

The electrophoretic mobility of oil droplets, also reported as ζ-potential, was measured by phase-analysis light scattering (PALS) with a Zetasizer NanoZS laser diffractometer (Malvern Instruments Ltd, Worcestershire, UK). It determines the surface electrical charge of the droplets dispersed in the continuous phase.

2.3.2. Viscosity

Viscosity of emulsions and nanoemulsions was measured from approximately 10 mL of sample using a SV-10 vibro-viscometer (A&D Company, Tokyo, Japan) vibrating at 30 Hz and constant amplitude. The viscosity of the sodium alginate solution (30 mPa s) was considered the viscosity of the dispersant material with respect to the dynamic light scattering measurements.

2.3.3. Whiteness index

The color of emulsions and nanoemulsions was measured with a Minolta CR-400 colorimeter (Konica Minolta Sensing, Inc., Osaka, Japan) at room temperature set up for illuminant D65 and 10° observer angle and calibrated with a standard white plate. CIE L^*, a^* and b^* values were determined and the Whiteness Index (WI) was

Table 1
Essential oil composition, oil refractive index and absorbance ($\lambda = 633$ nm) of essential oils.

Essential oil	Plant	Composition	Refractive index	Abs_{633}
Lemongrass	*Cymbopogon citratus*	Geranial, neral, geraniol	1.453	0.022
Clove	*Eugenia caryophyllata*	Eugenol, eugenil acetate	1.499	0.023
Tea Tree	*Melaleuca alternifolia*	4-terpineol, terpinene	1.465	0.001
Thyme	*Thymus vulgaris*	Timol, p-cimene	1.471	0.094
Geranium	*Pelargonium graveolens*	Citronelol, geraniol	1.462	0.157
Marjoram	*Origanum majorana*	4-terpineol, cis tuyan-4-ol	1.472	0.002
Palmarosa	*Cymbopogon martinii*	Geraniol, geranil acetate, linalol	1.461	0.003
Rosewood	*Aniba rosaedora*	α-terpineol, linalol	1.468	0.002
Sage	*Salvia officinalis*	Canfor, 1,8-cineol, canfene, sabinil acetate	1.479	0.071
Mint	*Mentha spicata*	Mentol	1.477	0.002

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