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Long-term stability of food-grade nanoemulsions from high methoxyl pectin containing essential oils

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ABSTRACT

Nanoemulsions have shown potential advantages over conventional emulsions due to their large active surface area, but are also susceptible to destabilization. Therefore, the purpose of this work was to assess the long-term stability (56 days) of nanoemulsions containing EOs (oregano, thyme, lemongrass or mandarin) stabilized by high methoxyl pectin and a non-ionic surfactant (Tween 80). The initial droplet size of nanoemulsion was below 50 nm regardless the EO type, which was confirmed by Transmission Electron Microscopy (TEM). Lemongrass and mandarin nanoemulsions remained optically transparent over time (56 days) and their droplet sizes were in the nano-range (between 11 and 18 nm), whereas the droplet size of oregano and thyme nanoemulsions increased up to 1000 nm probably due to Ostwald ripening. This fact induced creaming and a higher whiteness index in the latter nanoemulsions. The electrical charge (æ-potential) of nanoemulsions was negative due to the anionic nature of pectin molecule adsorbed at the oil-water interface, ranging between −6 and −15 mV depending on the EO type. However, lemongrass and mandarin nanoemulsions exhibited a more negative æ-potential than thyme or oregano EO indicating a stronger adsorption of pectin at the oil surface, and therefore a higher stability. The viscosity of nanoemulsions remained practically constant between 20 and 24 mPa s, during storage for all EOs. This work represents the starting point for future applications of nanoemulsions containing EOs to be incorporated in food products due to their high long-term stability.

1. Introduction

Essential oils (EOs) are natural compounds found in aromatic plants and herbs as secondary metabolites that present antioxidant and antimicrobial activity and also have been widely used as functional ingredients in food as flavorings (Burt, 2004). However their incorporation in food products presents several limitations due to their low solubility and intense aroma at high concentrations (Sánchez-González, Vargas, González-Martínez, Chiralt, & Cháfer, 2011). The emulsification of EO is currently used for their dispersion into food products but their functionality and long-term stability largely depends on the oil droplet size and distribution (Tadros, Izquierdo, Esquena, & Solans, 2004). In this sense, nanoemulsions can be used as carriers of lipophilic bioactive compounds for their incorporation in food products. Nanoemulsions consist of at least one immiscible liquid dispersed in another with a surfactant (nonionic or polymeric) in the form of small droplets, with an average droplet size between 20 and 200 nm (Burguera & Burguera, 2012; Solans, Izquierdo, Nolla, Azemar, & Garcia-Celma, 2005; Wulff-Pérez, Torcello-Gómez, Gálvez-Ruíz, & Martín-Rodríguez, 2009). Nanoemulsions exhibit several advantages over conventional emulsions (Qian & McClements, 2011; Tadros, Izquierdo, Esquena, & Solans, 2004). First, they are optically transparent so they might be good candidates to be incorporated in clear drinks or beverages (Qian & McClements, 2011). Second, nanoemulsions are kinetically stable colloidal systems (Solans et al., 2005). Third, they present a high active surface area thus having a potentially higher functionality (Qian & McClements, 2011). There are several methods to form nanoemulsions, but high-energy methods are the most commonly used. They require specialized mechanical devices such as high-pressure homogenizers and ultrasounds capable of generating intense mechanical disruptive forces inducing the breakup of the oil droplets (Mason, Wilking, Meleson, Chang, &

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Graves, 2006; Tadros et al., 2004). Nevertheless, nanoemulsions are susceptible to destabilization phenomena, so an optimal formulation is crucial for their long term stability (Henry, Fryer, Frith, & Norton, 2009; Mirhosseini et al., 2008). Stability may be defined as the resistance to physical changes. It has been reported that emulsions are destabilized via two mechanisms, being coalescence and Ostwald ripening (Henry et al., 2009; Nazarzadeh, Anthonypillai, & Sajjadi, 2013; Solans et al., 2005). Coalescence occurs when two oil droplets contact due to the weak stearic repulsion between them, and they unify in a sole larger droplet. Ostwald ripening is due to the diffusion of oil molecules from small to large droplets through the continuous phase in relatively water-soluble oils (such as EOs), leading to an increase in the oil droplet size (Nazarzadeh et al., 2013; Rao & McClements, 2012).

On the other hand, food hydrocolloids such as polysaccharides and proteins have been used to stabilize emulsions in several studies (Ye & Singh, 2006). Pectin is a naturally-sourced polysaccharide and is commonly used in the food and pharmaceutical industries as gelling and thickening agent (Liu, Fishman, & Hicks, 2007). In addition, high methoxyl pectin can be used as emulsifying agent (Liu et al., 2007; Mirhosseini et al., 2008; Pérez-Espitia, Du, Avena-Bustillos, Ferreira Soares, & McHugh, 2014; Sungthongjeen, Sriamornsak, Pitaksuteepong, Somsiri, & Puttipipatkhachorn, 2004). Pectins have amphiphilic character that helps to reduce the interfacial tension between oil and water phases and can be effective and suitable in the preparation and formulation of emulsions (Burapapadh, Kumpugdee-Vollrath, Chantasart, & Sriamornsak, 2010). Therefore, the aim of the present work was to study the stability of food-grade nanoemulsions containing essential oils (oregano, thyme, mandarin and lemongrass) to determine what type of nanoemulsions remained without changes during the storage time. For this purpose, we evaluated the physicochemical characteristics and overall long-term stability (56 days at room temperature) of the nanoemulsions.

2. Material and methods

2.1. Materials

Essential oils from oregano (*Origanum compactum*) and thyme (*Thymus vulgare*) were purchased from Dietetica Intersa (Spain), whereas lemongrass (*Cymbopogon citratus*) was obtained from Laboratories Dicana (Spain). Mandarin EO (*Citrus reticulata*) was kindly donated by Indulleida, S.A. (Spain). Food-grade high methoxyl pectin (Unipectine QC100 from citrus source) was provided by Cargill Inc. (Spain). Tween 80 (Polyoxyethylenesorbitan Monoesterate) (Lab Scharlab, Spain) was used as food-grade non-ionic surfactant. Ultrapure water, obtained from Millipore Milli-Q filtration system (0.22ìm) was used for the formulation and analysis of nanoemulsions.

2.2. Primary emulsion formation

The independent variables used were: emulsion formulation and oregano, thyme, lemongrass and mandarin essential oils. High methoxyl pectin (1% w/v) was dissolved in water at 80–85 °C, with continuous stirring until it was completely dissolved and the solution was cooled down to 25 °C. A primary emulsions was made mixing the pectin aqueous solution and the essential oil (2% v/v) and Tween 80 (5% w/v) by means of a laboratory T-25 digital Ultraturrax (IKA, Staufen, Germany) working at 9500 rpm for 2 min. The final volume of the primary emulsion was 1000 mL. Droplet size and droplet size distribution, Z-potential, creaming index, color, viscosity and transmission electron microscopy were measured and were considered as dependent variables (Table 1).

2.3. Nanoemulsion formation

Nanoemulsions were obtained by microfluidization (M-110P, Microfluidics, USA) at 150 MPa for 5 cycles. Nanoemulsions were cooled down at the outlet of the microfluidization unit through an external coil immersed in a water bath with ice, so that temperature was kept at 10 °C. The final volume of each nanoemulsion was 950 mL, as 50 mL were discarded to avoid the dilution of the sample. For stability studies, aliquots of nanoemulsions were placed in plastic test tubes with caps and stored at room temperature (~25 ± 2 °C) in the absence of light. Analytical determinations were performed right after preparation and along storage time (0, 7, 14, 21, 28, 35, 42, 49 and 56 days). When creaming occurred, nanoemulsions were homogenized to re-disperse the cream layer before the analysis.

2.4. Nanoemulsion characterization

2.4.1. Droplet size and droplet size distribution

The average droplet size of the nanoemulsion was determined by dynamic-light-scattering (DLS), using a Zetasizer Nano-ZS laser diffractometer (Malvern Instruments Ltd., Worcestershire, UK), working at 633 nm, equipped with a backscatter detector (173°), which is used to specifically measure sub-micron particles. The DLS measures particle diffusion moving under Brownian-motion. Nanoemulsions were diluted 100 times with milli-Q water to avoid multiple-scattering effect and stirred to ensure sample homogeneity. DTS0012-disposable cuvettes were used, which the minimum volume sample is required (1 mL). The refractive indexes (RI) of the oil phases were measured with a manual refractometer (model J357, Rudolph research, New Jersey, USA) being 1.501, 1.497, 1.484 and 1.475 for the oregano, thyme, lemongrass and mandarin EOs, respectively. The absorbance of the EOs at 633 nm was measured with a spectrophotometer Cecil CE 1021 (Cambridge, England) being 0.002, 0.002, 0.024 and 0.004 for the oregano, thyme, lemongrass and mandarin EOs, respectively. Droplet-size measurements are reported as average-volume. Polydispersity Index (PdI) was also recorded, a value near 1 indicates a heterogeneous or multimodal distribution of droplet sizes. Determinations were performed at 25 °C, 24 h after the preparation of the nanoemulsions and every 7 days up to 56 days.

2.4.2. Transmission electron microscopy

Nanoemulsions were observed by negative-staining electron microscopy as a direct measure of their droplet size and shape. The sample was adsorbed onto carbon film on 300 mesh copper grids for 1 min. Then, the grid was washed by floating it face-down on a drop of Milli-Q ultrapure water for 1 min. This process was repeated three times. Finally, the sample was negatively stained by floating the grid face-down on a drop of 2% (w/v) ammonium molybdate at pH 6.2 for 1 min. The images of the samples were obtained observing the grids in a Jeol-JEM 1010 transmission electron microscope (Biodirect, Inc., Massachusetts, USA) at an acceleration voltage of 100 kV.

2.4.3. Particle charge measurements (ζ-potential)

The electrical charge (æ-potential) of the oil droplets in the nanoemulsions was determined by phase-analysis light scattering (PALS) measuring their electrophoretic mobility using an automated capillary electrophoresis device (Zetasizer Nano ZS series, Malvern Instruments Ltd, Worcestershire, UK) and working at 633 nm laser at 25 °C. Sample was placed in a disposable zeta cuvette that acted as a measurement chamber, and the æ-potential was determined by measuring the particle's direction and velocity when an electric field was applied. The Smoluchowski model was