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Influence of essential oils and pectin on nanoemulsion formulation: A ternary phase experimental approach

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ABSTRACT

A pseudo-ternary phase experimental approach was used to model the influence of the mixture components concentration on the nanoemulsions properties as ternary systems. For this, several types of essential oils (EO) were used as lipid phase, being oregano (OR-EO), thyme (TH-EO), lemongrass (LG-EO) and mandarin (MN-EO), while pectin and Tween 80 were studied as emulsifiers. All formulations were processed by microfluidization at 150 MPa and 5 cycles. Polynomial models were fitted to experimental data and their adjusted R^2 and p-values were obtained. Remarkably, a pectin concentration of 1% (*w/w*) allowed the formation of submicron emulsions between 350 and 850 nm in the absence of Tween 80 for all the studied EOs, thus confirming its emulsification capacity. In general, increasing the pectin concentration up to 2% (*w/w*) enlarged the particle size of emulsions and their viscosity thus suggesting decreased emulsification efficiency during microfluidization. Nonetheless, nanoemulsions with particle sizes below 500 nm were obtained when a minimum Tween 80 concentration of 1.8% (*w/w*) was used, regardless the pectin or EO concentrations. The modest decrease in the ζ-potential that was observed depending on the type of EO at increasing pectin concentrations indicated that pectin is not or weakly adsorbed at the oil-water interface. All nanoemulsions were transparent at high surfactant and low EO concentrations due to a weak light scattering of the nano-sized oil droplets. Thus, this work contributes in elucidating the role of pectin and small molecule non-ionic surfactants on the formation of submicron emulsions and nanoemulsions containing essential oils.

1. Introduction

Essential oils (EOs) are natural compounds that contain a complex mixture of terpenoids, with non-volatile and volatile nature produced by aromatic plants as secondary metabolites (Fisher & Phillips, 2008; Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2014). EOs have been traditionally used as natural flavorings and more recently as natural antimicrobials for food preservation (Guerra-Rosas, Morales-Castro, Ochoa-Martínez, Salvia-Trujillo, & Martín-Belloso, 2016). Due to their lipophilic nature, they are able to interact with biological membranes of microbial cells causing the leakage of cytoplasmatic content and the subsequent cell collapse (Burt, 2004; Guerra-Rosas et al., 2016). Besides the benefits of adding EOs to food matrices, their poor water solubility, their intense aroma or their potential toxicity at high concentrations needs consideration (Svoboda, Brooker, & Zrustova, 2006). Therefore, the design of adequate delivery systems able to encapsulate, protect and release lipophilic bioactive compounds into food matrices more efficiently represents a challenge for the food technology field.

Recently, nanoemulsions have been described as colloidal dispersions of oil droplets with particle size diameters lower than 500 nm, which are suspended within an aqueous phase (Otoni, Avena-Bustillos, Olsen, Bilbao-Sáinz, & McHugh, 2016). Nanoemulsions seem to be a promising tool for incorporating antimicrobial EOs in foods and they have been reported to present several potential advantages in comparison with conventional emulsions. Nanoemulsions present a higher active surface area/volume ratio due to their small droplet size, thus enhancing the transport of active compounds through biological membranes. Therefore, the use of nanoemulsions as carriers of antimicrobial essential oils

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would allow reducing the concentration to be used in order to achieve equivalent microbial inactivation levels of those of conventional emulsions or bulk oils. This, would help overcoming the low threshold values of essential oil incorporation for consumer acceptance (McClements, 2012; Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2014a; Solans, Izquierdo, Nolla, Azemar, & Garcia-Celma, 2005).

The fabrication of nanoemulsions requires the use of surfactants in order to stabilize the oil droplets in the aqueous phase. Surfactants are able to adsorb at the oil-water interface thus lowering the interfacial tension of oil in water, which facilitates the emulsification process and prevents different destabilization phenomena such as aggregation, flocculation or coalescence (Kralova & Sjöblom, 2009). In addition, natural biopolymers are gaining importance for their use as emulsifiers and thickening agents. The increase in viscosity of the aqueous phase prevents the destabilization of emulsions and nanoemulsions due to a diminished gravitational movement of oil droplets, which may retard or avoid droplets coalescence (Guerra-Rosas et al., 2016). However, some biopolymers may also present surface active properties thus having emulsifying capacity. For instance, pectin is a natural biopolymer mainly present in fruits and vegetables that has shown certain adsorption capacity at oil-water interfaces and may enhance the stability of emulsions (Alba & Kontogiorgos, 2017; Chan, Choo, Young, & Loh, 2017; Ozturk & McClements, 2016). The lipid fraction also plays an important role in the physicochemical properties, which depend on the characteristics of the different EOs including their chemical composition and the hydrocarbon chain length (Hopkins, Chang, Lam, & Nickerson, 2015). In fact, it is reported that short chain fatty acids, such as EOs, are prone to destabilization phenomena due to Oswald ripening effect since they consist on an aromatic carbon ring, which makes them slightly water-soluble oils (Suriyarak & Weiss, 2014). In this context, differences between nanoemulsion stability incorporating different EOs may be rather affected by their chemical composition (Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2015).

The selection of the appropriate concentration of each individual ingredient in the formulation of nanoemulsions is of crucial importance in order to obtain systems with the desired physicochemical characteristics (Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2014b). Building conventional pseudo-ternary phase diagrams has been explored as a strategy to optimize the formulation of multi-component emulsions. However, determining the region where nanoemulsions are formed requires performing a large number of experimental combinations and formulations. Oppositely, the ternary phase experimental design, and specifically D-optimal models, is a methodology which allows to evaluate the effect of multiple factors, alone or in combination, with a minimum number of experiments (Ramsey, 1997; Salvia-Trujillo et al., 2014b). Consequently, the combination of pseudo-ternary phase diagrams and response surface methodologies can improve the understanding of the influence of the emulsion components concentration and their interactions as well as predicting optimized multi-component formulations with controlled physicochemical and functional properties (Ren, Mu, Alchaer, Chtatou, & Müllertz, 2013). To the best of our knowledge, there is a lack of research works using this strategy for the formulation of nanoemulsions containing several emulsifying agents, such as Tweens and pectin. This would allow to fully map the behavior of the different components and their interactions on nanoemulsion characteristics.

Thus, the aim of this work was to study the influence of four different essential oils (EOs) such as oregano (OR-EO), thyme (TH-EO), lemongrass (LG-EO) and mandarin (MN-EO) essential oils on the properties (oil droplet size, ζ-potential, viscosity and whiteness index) of nanoemulsions. Moreover, the use of a small molecule surfactant (Tween 80) and a natural biopolymer (pectin) were studied as emulsifiers.

2. Material and methods

2.1. Materials

Oregano (Origanum compactum), thyme (Thymus vulgare) and lemongrass (Cymbopogon citratus) essential oils (EOs) were purchased from Essential'aroms® (Dietetica Intersa, Lleida, Spain) and had a 100% purity. Oregano EO was composed of 26–45% carvacrol, 9–30% thymol, 9–26% *p*-cymene, 12–20% γ-terpinene and traces of α-terpinene and β-mircene. Thyme EO mainly contained thymol (30–47%) and *p*-cymene (15–35%) with traces of carvacrol and linalool. Lemongrass had 39–47.1% of geranial, 28.9–35.7% of neral, 0.5–8.3% of limonene, 0.9–6.9% of geranyl acetate, 0–5.3% of geraniol and traces of citronelol, eugenol and linalool among others. Mandarin EO (Citrus reticulata) 100% pure, was kindly donated by Indulleida, S.A. (Lleida, Spain) and contained 74.4% of D-limonene, a 13.6% of oxygenated monoterpenes and traces of *cis*-oxide limonene, *cis-para*-mentha-2,8-dien-1-ol, carvone, *trans*-carveol and z-patchenol (>1%). Food-grade high methoxyl pectin (Unipectine QC100 from citrus source) with a degree of methylesterification (DM) from 69 to 75% and a particle size of the dry powder at least 99% less than 315 μm (ASTM Screen N°45) was kindly provided by Cargill Inc. (Reus, Spain). Tween 80 (Polyoxyethylenesorbitan Monoesterate) (Lab Scharlab, Barcelona, Spain) was used as food-grade non-ionic surfactant. Ultrapure water, obtained from Millipore Milli-Q filtration system (0.22 μm) was used for the formulation and analysis of nanoemulsions.

2.2. Pseudo-ternary phase experimental design

An experimental mixture design, specifically a D-optimal design, was used to study the influence of the essential oil (EO), Tween 80 and pectin concentrations on the oil droplet diameter (nm), ζ-potential (mV), viscosity (mPa·s) and color of emulsions. For the experimental design, the software Design Expert 7.0.0 (Stat Ease Inc., Minneapolis, MN) was used. Posteriorly, pseudo-ternary phase diagrams were built in order to predict and identify the regions where stable nanoemulsions are formed. Firstly, for the experimental design setup, the concentration of each component was set according to the following constraints expressed as weight fraction in the aqueous phase (% *w/w*): $0.12 \leq EO \leq 1$ in case of OR-EO and TH-EO but $0.1 \leq EO \leq 2$ for LG-EO and $0.05 \leq EO \leq 3$ in case of MN-EO; $0 \leq$ Tween 80 ≤ 6, $1 \leq$ pectin ≤ 2 and for all the cases: EO + Tween 80 + pectin + water = 1. The minimum concentration of EO was set above its minimum inhibitory concentration against the most common pathogenic microorganisms that proliferate in foods such as *Escherichia coli, Listeria monocytogenes or Staphylococcus aureus* (Burt, 2004; Hammer, Carson, & Riley, 1999), whereas the maximum concentration was set regarding the toxicity of EOs at high concentrations (Svoboda et al., 2006). Tween 80 concentration was set to reach a maximum oil:surfactant ratio of 1:3 (expressed in weight), which ensures that enough surfactant molecules are available to be adsorbed at the oil-water interface (Qian & McClements, 2011; Salvia-Trujillo et al., 2014b). Pectin is generally recognized as safe (GRAS) thus its only limitation regarding its addition to nanoemulsions is the increase of viscosity of the mixtures in order to pass through the microfluidizer (Laurent & Boulenguer, 2003).

After running the statistical software, a series of mixture combinations were generated as an output of the D-optimal design. All oil-in-water emulsions were prepared in random order and