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Fabrication, stability and efficacy of dual-component antimicrobial nanoemulsions: Essential oil (thyme oil) and cationic surfactant (lauric arginate)

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ABSTRACT

The influence of a cationic surfactant (lauric arginate, LAE) on the physical properties and antimicrobial efficacy of thyme oil nanoemulsions was investigated. Nanoemulsions prepared from pure thyme oil were highly unstable due to Ostwald ripening, but they could be stabilized by adding a ripening inhibitor (corn oil) to the oil phase prior to homogenisation. The loading capacity and antimicrobial efficacy of thyme oil nanoemulsions were significantly increased by adding LAE. In the absence of LAE, at least 60 wt% corn oil had to be added to the lipid phase to inhibit Ostwald ripening; but in the presence of 0.1 wt% LAE, only 30 wt% corn oil was needed. LAE addition substantially increased the antimicrobial efficacy of the thyme oil nanoemulsions: 200 μg/ml thyme oil was needed to inhibit growth of a spoilage yeast (*Zygosaccharomyces bailii*) if LAE was added, whereas ⩾400 μg/ml was needed in the absence of LAE.

1. Introduction

Essential oils are natural compounds which can act both as flavouring agents and antimicrobial agents, and therefore they have been widely used as functional ingredients in food, cosmetic, and pharmaceutical applications (Bakkali, Averbeck, Averbeck, & Waomar, 2008). Essential oils contain a complex mixture of non-volatile and volatile compounds. Commercial essential oils are often a mixture of different constituents that vary in their molecular and physicochemical properties, for example, molecular weight, water solubility, polarity, and biological activity. The major molecular constituents within commercial essential oils can be classified into three broad classes: phenols, terpenes, and aldehydes (Bakkali et al., 2008; Burt, 2004; Ceylan & Fung, 2004). Many essential oils have been shown to exert strong antibacterial, antiviral, and antifungal activities (Burt, 2004; Ferreira et al., 2010; Giatrakou, Ntzimani, & Savvaidis, 2010), leading to their application as natural antimicrobial additives to extend the shelf life of food and beverage products. For example, thyme oil has been shown to have inhibitory activities against various bacteria and yeasts (Gaysinsky, Davidson, McClements, & Weiss, 2008). Thymol, the primary component of thyme oil (Gaysinsky, 2007), has also been reported to exhibit antimicrobial activity against many bacteria and fungi (Friedman, Henika, & Mandrell, 2002; Sivropoulou et al., 1996). The fact that essential oils are considered to be "natural" components makes them highly desirable for application in many commercial food and beverage products, since there is growing consumer demand for natural rather than synthetic additives.

However, essential oils are hydrophobic compounds and usually have quite low solubility in water, which limits their utilisation in aqueous-based foods and beverages. This problem could be simply resolved by encapsulating essential oil within emulsion-based delivery systems (Chang, McLandsborough, & McClements, 2012; Donsi, Annunziata, Sessa, & Ferrari, 2011; Donsi, Cuomo, Marchese, & Ferrari, 2014; Salvia-Trujillo, Rojas-Grau, Soliva-Fortuny, & Martin-Belloso, 2013, 2014; Wu, Lin, & Zhong, 2014; Ziani, Chang, McLandsborough, & McClements, 2011). After essential oils are encapsulated into suitable emulsion delivery systems, they can then be incorporated into aqueous-based foods (e.g., beverages) and other products by simple mixing. Based on their droplet size, emulsions may be divided into

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conventional emulsions (diameter > 200 nm) or nanoemulsions (diameter < 200 nm) (McClements, 2011, 2012; McClements & Rao, 2011). The diameter of the nanoemulsion droplets is often much smaller than the wavelength of light ($d \ll \lambda$), therefore the nanoemulsions are often transparent or only slightly turbid, since they do not scatter light strongly. This property is beneficial, making nanoemulsions suitable for transparent products, such as clear beverages, sauces, soups, and syrups. The small dimensions of the nanoemulsion droplets also mean that they typically have much better physical stability against gravitational separation, flocculation, and coalescence than conventional emulsions (Mason, Wilking, Meleson, Chang, & Graves, 2006; McClements, 2011; McClements & Rao, 2011; Tadros, Izquierdo, Esquena, & Solans, 2004). In addition, as droplets size decreases, the biological activity of the encapsulated active compounds within emulsions or nanoemulsions often increases (Acosta, 2009; Hatanaka et al., 2010; Huang, Yu, & Ru, 2010). Therefore, in many cases it is beneficial to encapsulate functional components into nanoemulsions, compared to conventional emulsions.

A major limitation to formulating antimicrobial nanoemulsions containing essential oils is that they have some water-solubility, which means that the oil droplets are prone to Ostwald ripening (Chang et al., 2012; Ziani et al., 2011). Ostwald ripening (OR) is the growth of large oil droplets at the expense of smaller oil droplets due to diffusion of oil molecules through the intervening aqueous phase (Kabalnov, 2001; Kabalnov & Shchukin, 1992; Wooster, Golding, & Sanguansri, 2008). The driving force for OR is the fact that the solubility of oil in the immediate vicinity of an oil droplet increases as the droplet diameter decreases. The Ostwald ripening rate usually increases with increasing solubility of the oil phase in the water phase (McClements, 2005, 2011; McClements & Rao, 2011; Wooster et al., 2008). OR can be prevented by incorporating sufficient amounts of highly water-insoluble oils in the droplets, since this generates an entropy of mixing effect that counteracts the imbalance of droplet size effect (Kabalnov & Shchukin, 1992; Wooster et al., 2008). These water-insoluble oils are typically referred to as "ripening inhibitors", and are usually highly non-polar substances with relatively high molecular weights, such as corn oil (Chang et al., 2012; McClements, Henson, Popplewell, Decker, & Choi, 2012; Ziani et al., 2011), sunflower oil (Donsi, Annunziata, Vincensi, & Ferrari, 2012), and medium chain triglycerides (MCT) (Liang et al., 2012; Terjung, Loffler, Gibis, Hinrichs, & Weiss, 2012). In our previous study, we showed that Ostwald ripening of thyme oil nanoemulsions could be inhibited by mixing thyme oil with sufficiently high amounts of water-insoluble oils (e.g., $\geqslant$60 wt% corn oil in the lipid phase) before homogenisation, (Chang et al., 2012; Ziani et al., 2011). This method can therefore be used to inhibit Ostwald ripening in nanoemulsions, provided that a sufficiently high quantity of water-insoluble oil is utilised.

Lauric arginate (LAE) is a food-grade cationic surfactant that is a highly potent antimicrobial active against a wide range of food pathogens and spoilage organisms (Brandt et al., 2010; Dai, Normand, Weiss, & Peleg, 2010; Theinsathid, Visessanguan, Kruenate, Kingcha, & Keeratipibul, 2012). In this study, we investigated the ability of LAE to improve both the physical stability and antimicrobial activity of thyme oil nanoemulsions. In particular, we hypothesized that utilising two different antimicrobial agents within a single nanoemulsion (LAE and thyme oil) may lead to synergistic antimicrobial activity. An acid-resistant spoilage yeast (*Zygosaccharomyces bailii*) was used as a model microorganism to assess the antimicrobial activity of the nanoemulsions. The results of this study have important implications for the design and utilisation of nanoemulsions as antimicrobial delivery systems in the food and other industries.

2. Materials and methods

2.1. Materials

Thyme oil was obtained from Optimal Health Solutions (La Pine, Oregon). Corn oil was purchased from a local grocery store. A non-ionic surfactant (Tween 80, T80) was purchased from Sigma–Aldrich Co. (St. Louis, MO), and the cationic surfactant lauric arginate (LAE) (MIRENAT-P/100) was provided by Grupo Lamirsa (Terrassa, Spain) which was reported to contain 85 wt% LAE.

2.2. Nanoemulsion preparation

To made thyme oil nanoemulsions, we first prepared aqueous phase and oil phase separately. The aqueous phase used to prepare the nanoemulsions consisted of 1.0 wt% Tween 80 or 0.9 wt% Tween 80 + 0.1 wt% LAE dispersed in an aqueous buffer solution (5 mM citrate buffer, pH 3.5). Lipid phases were prepared by mixing different mass ratios of thyme oil and ripening inhibitor (corn oil) prior to homogenisation. The lipid phase (10% w/w) was mixed with the aqueous phase (90% w/w) using a high-speed blender for 2 min. The resulting crude emulsion was then homogenised by passing it five times through a high pressure homogeniser at 10 kPa (Microfluidics 110L, Microfluidics Corp., Newton, MA, USA) to further reduce the particle size. After preparation, the nanoemulsions formed were stored at 4 °C prior to analysis.

2.3. Particle size measurements

The mean particle diameters (*Z*-averages) of the nanoemulsions were measured using a dynamic light scattering instrument (Zetasizer Nano ZS, Malvern Instruments, Worcestershire, UK), following the method described in our previous publication (Chang et al., 2012).

2.4. Particle charge measurements

The electrical charge (ζ-potential) of the droplets in the nanoemulsions was measured using a particle electrophoresis instrument (Zetasizer Nano ZS, Malvern Instruments, Worcestershire, UK), following the method as described previously (Chang et al., 2012).

2.5. Yeast strain

An acid resistant spoilage yeast, *Z. bailii* (ZB), was used as a target microorganism, to examine the antimicrobial effects of different nanoemulsions. The strain was obtained from the Pepsico R&D Culture Collection (Valhalla, NY). The yeast strain was refreshed and cultured according to the method described by us previously (Chang et al., 2012), and then diluted to about 10^6 CFU/ml in MEB media (pH 3.5, 5 mM citrate buffer) to conduct the following antimicrobial assay.

2.6. Determination of antimicrobial activity

All nanoemulsions were filtered sterilized using 0.45 μm polyethersulfone membrane filters (F2500-14, Thermo Scientific, Germany) prior to carrying out the antimicrobial activity assays. The particle size distributions of the nanoemulsions did not change after filter sterilization (data not shown), which indicated that all the droplets passed through the filter.

Appropriate amounts of sterile thyme oil nanoemulsions were added to MEB media (pH 3.5, 5 mM citrate buffer), to make incubation media containing a serial of antimicrobial nanoemulsions. The