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Formation, optical property and stability of orange oil nanoemulsions stabilized by *Quallija* saponins

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ABSTRACT

Nanoemulsions have the potential to deliver insoluble materials and yet provide a transparent appearance in clear beverage applications. The influence of oil phase composition, phase viscosity and homogenization conditions on the formation, stability and optical properties of orange oil-based nanoemulsions was investigated. The nanoemulsions were formed using *Quillaja* saponins (QS) and homogenization accomplished using Microfluidization®. The results indicated oil phase composition and viscosity ratio between phases are key parameters determining the mean droplet diameter (MDD) of the prepared nanoemulsions. Orange oil nanoemulsions stabilized by QS showed MDD as small as 67 nm corresponding to turbidity of 71 NTU at dispersed phase concentration of 0.5 mg/g. Lipid phase refractive index showed no effect on turbidity of orange oil nanoemulsions at MDD < 150 nm, while MDD was linearly related to the turbidity with MDD ranging from 60 nm to 120 nm. Ostwald ripening was identified as main destabilization mechanism of orange oil nanoemulsions stabilized by QS at the early stage of storage. The rate of Ostwald ripening was reduced with increasing amounts of medium chain triglycerides (MCT) in the dispersed phase. An insoluble material, e.g., ester gum or MCT, was required to form stable orange oil-based nanoemulsions.

1. Introduction

In the past ten years, nanoemulsions have become an active research area in the food and flavor industries due to their unique properties, such as improved bioavailability of actives and transparent appearance (McClements, 2011; McClements & Rao, 2011; Zhang, Peppard, & Reineccius, 2015). Flavor nanoemulsions offer an attractive feature of being optically transparent which is of particular interest for beverages and alcohol-free mouthwash products. Most of the relevant information is in patents on short chain polyols/alcohol-based microemulsions (Chanamai, 2007; Skiff, Baaklini, & Vlad, 2007, Schultz & Monnier, 2013). Recently, a great deal of work has been reported on food-grade nanoemulsions by McClements and his co-workers (Chang & McClements, 2014; Rao & McClements, 2012; Yang, Marshall-Breton, Leser, Sher, & McClements, 2012). The major challenges in producing flavor nanoemulsions are two fold: 1) to produce nanoemulsions with high efficacy using food-grade emulsifiers without undesirable taste; and 2) to stabilize nanoemulsions against Ostwald ripening.

Quillaja saponins (QS) have gained attention in recent years because of their properties as foaming agents in beverages and emulsifiers in foods, as well as their applications in cholesterol-reduction and flavor enhancement (Nayyar, Schulok, Saleeb, & Modesta, 1998; Ozturk, Argin, Ozilgen, & McClements, 2014; San & Briones, 1999). QS are obtained by aqueous extraction of the milled bark or whole wood of *Quillaja saponaria* Molina. *Quillaja* extract contains over 100 saponins (Kite, Howes, & Simmonds, 2004). The basic structure consists of glycosides linked to triterpene aglycones. The hydrophilic groups of the molecule consist of sugars such as rhamnose, xylose, arabinose, galactose, fucose, and glucuronic acid, while the hydrophobic portions of saponins are comprised of quillaic acid and other acids (Kensil, Soltysik, & Marciani, 1996; Setten & Werken, 1996). Glucuronic acid is the only ionizable group on the molecule; the other acids are attached as ester bonds to the main structure (Mitra & Dungan, 1997). Recently, purified *Quillaja* extract has been commercialized as a natural alternative to gum arabic. This natural surfactant appears to provide a new opportunity for producing nanoemulsions.

Since most essential oils have considerable water solubility, Oswald ripening is the major mechanism leading to physical

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instability. Incorporation of ester gum and triglyceride in the oil phase to inhibit Ostwald ripening and improve nanoemulsion stability has been reported by a few authors (Lim et al., 2011; McClements, Henson, Popplewell, Decker, & Choi, 2012; Wooster, Golding, & Sanguansri, 2008). It was found that Ostwald ripening was inhibited by using a large molar volume of triglyceride oils in the dispersed phase. Triglyceride oils are water insoluble so they will not contribute to Oswald ripening in themselves. However, by providing a significant mass of very hydrophobic material, the actives (e.g., flavorings which have adequate solubility in the continuous phase to be problematic) will preferentially partition into this mass thereby minimizing the ability of the flavoring to migrate to larger droplets. An alternative approach was suggested by modification of the oil/water interface to inhibit Ostwald ripening of hydrocarbon-based emulsions (Mun & McClements, 2006). It was suggested that the formation of a thick, dense interfacial membrane provides mechanical resistance to shrinkage and growth of oil droplets that normally occurs during Ostwald ripening.

The primary purpose of the present study was to evaluate oil phase composition on formation and stability of orange oil-containing nanoemulsions. The impacts of droplet size and oil phase refractive index on optical properties (i.e., turbidity) were also investigated. This study has provided a practical understanding of the formulation of flavor nanoemulsions in applications of clear beverage and alcohol-free mouthwash products.

2. Materials and methods

2.1. Materials

Quillaja saponins (Q-Naturale™ 200, QS) were donated by Ingredion (Westchester, IL, USA). Cold pressed Valencia orange oil was obtained from Citrus and Allied Essences Ltd. (Lake Success, NY, USA) and Miglyol®812, a medium chain triglyceride (MCT), was purchased from Sasol (Houston, TX, USA); ester gum was obtained from J.H. Calo Co. (Westbury, NY, USA). Miglyol 812 is a combination of triglycerides based on the following fatty acid composition: $C_{6:0}$ max. 0.02 g/g; $C_{8:0}$ 0.5–0.65 g/g; $C_{10:0}$ 0.3–0.45 g/g; $C_{12:0}$ max. 0.02 g/g and $C_{14:0}$ max. 0.01 g/g (Sasol, Houston, TX, USA). The cold pressed Valencia orange oil used was comprised primarily of limonene (0.95 g/g), myrcene (19 mg/g), sabinene (2.4 mg/g), α-pinene (4.2 mg/g), octanal (2 mg/g), geranial (1 mg/g), linalool (2.5 mg/g), decanal (2.8 mg/g), and oxygenated compounds (10 mg/g) as measured by gas chromatography. Citric acid and sodium citrate were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Preparation of nanoemulsions

A 10 mmol/L, pH 3.6 citrate buffer was used as the continuous phase to prepare nanoemulsions. Solutions of 0.06 g/g QS were prepared in this buffer by stirring for 2 h at room temperature. A mixed oil phase was prepared by blending orange oils with ester gum or MCT at different ratios (0–0.6 g/g in dispersed phase). Nanoemulsion concentrates were prepared by premixing 0.05 g/g oil phase and 0.95 g/g continuous phase containing QS using a high shear mixer (Greerco Corp., Hudson, NH) at 6000 rpm for 2 min. This coarse emulsion was passed through a Microfluidizer® (Model M–110Y, Microfluidics Corporation, Newton, MA) at 152 MPa for multiple passes depending upon the experiment. The nanoemulsions were maintained at about ambient temperature during multiple passes using a cooling coil and bath attached to the Microfluidizer® output line. All emulsions were produced in duplicates.

2.3. Measurement of viscosity

Viscosities of dispersed and continuous phases were measured using a Brookfield rotational rheometer (Brookfield, RVIII model, Stoughton, MA, USA) with cone and plate geometry. A series of standard solutions were used to calibrate the viscometer. In preliminary experimentation, different spindles and spindle ration speeds were tested. LV#61 spindle and speed of 30 rpm were selected for all samples based on the criteria that the torque reading should be between 5% and 85% in order to obtain accurate and reliable data recommended by Brookfield. The data were acquired via a personal computer using Rheocalc Software developed by Brookfield engineering lab. Two measurements were conducted for each sample and the average was reported.

2.4. Droplet size determination

Dynamic light scattering (DLS, BIC 90Plus, Brookhaven Instrument Corporation, NY, USA) was used to quantitatively determine the mean droplet diameter (MDD) and particle size distribution of the prepared nanoemulsions. It included a photometer equipped with an electrically heated silicon oil bath, Lexel 95-2 Ar + laser operating at a wavelength of 659 nm, Brookhaven BI-DS photomultiplier, and Brookhaven BI-9000AT correlator. The protocol for measurement and data acquisition were detailed elsewhere using the same instrument (Zhu, 2014). A brief summary is provided here. The light intensity correlation function was obtained at 25 °C with scattering angle of 90°. Water was selected as solvent with refractive index of 1.3330 and viscosity of 0.89 mPa s. The correlation function is a combination of the diffusion coefficient (D*i*) of each droplet which is converted to droplet diameter (d*i*) using the Stokes–Einstein equation (Eq. (1)),

$$d_i = \frac{k_b T}{3\pi\eta D_i} \tag{1}$$

where k_b is the Boltzmann constant, T is the absolute temperature, and η is viscosity. Correlation functions were downloaded from the Brookhaven ZetaPALS and fitted using REPES program (incorporated in the GENDIST software) developed by Jakes (1988, 1995). REPES yields a series of discrete particle diameters to represent the particle size distribution. The REPES algorithm was solved to provide the intensity weighted size distribution as defined in Eq. (2),

$$d_I = \frac{\sum n_i d_i^6}{\sum n_i d_i^5} \tag{2}$$

where n_i is the number of droplets with a diameter of d_i. The averaged diameter in mass, d*m*, is defined in Eq. (3),

$$d_m = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \tag{3}$$

All nanoemulsions were diluted to 0.5 mg/g dispersed phase before analysis using 10 mmol/L citrate buffer of pH 3.6. This pH was selected because the potential application of flavor nanoemulsions is for acidic beverages. Each sample was run for 6 cycles and Mass average mean droplet diameter (MDD) is reported in present study.

2.5. Cryo-transmission electron microscopy (Cryo-TEM)

Cryo-TEM (FEI Tecnai G2 F30, Hillsboro, OR, USA) was used to qualitatively characterize nanoemulsions. Nanoemulsions were diluted to about 0.5 mg/g of dispersed phase with 10 mmol/L citrate