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Influence of surfactant and oil composition on the stability and antibacterial activity of eugenol nanoemulsions

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ABSTRACT

Eugenol (Eu) nanoemulsions were prepared by high-pressure homogenization with two surfactants and varied oil compositions. Their stability and antibacterial activities were further evaluated. The results from dynamic light scattering demonstrated that the stability of bulk Eu emulsions stabilized by sodium dodecylsulfate (SDS) was worse than that by Tween 80 (T80). The improvement of emulsion stability by addition of bean oil (BO) depended on the interfacial surfactants. For SDS-stabilized emulsions, Eu emulsions would become stable with the increasing amount of BO. For T80-stabilized emulsions, the improvement of Eu emulsion stability was noticeably different as reported. With the increment of BO, Eu emulsions became stable first, then unstable and finally stable. The repeat instability occurred in Eu emulsions at 60 wt% BO. The results of antibacterial activity showed that emulsion encapsulation could protect and even enhance the antibacterial activity of Eu. The antibacterial capacity of Eu emulsions was affected by the surfactant type, listed as anionic > nonionic surfactant. Time-kill assay presented that lower BO content in Eu-T80 emulsions, the better effect was obtained on the bacterial inhibition against *E. coli*.

1. Introduction

Accompanied by growing consumer interest in natural food additives, the demand for effective antioxidants and antibacterial agents from natural resources as an alternative to suppress food deterioration and spoilage during processing and storage has been reinforced. Plant-derived essential oils have been widely utilized as natural antimicrobial agents in many industrial applications for a long history. It has been reported that they can effectively inhibit a wide range of bacterial growth and cause few side effects (Nair, Gopi, Mohankumar, Kavina, & Panneerselvam, 2011; Machado et al., 2011). Eugenol (Eu), a well-studied essential oil derived from clove, cinnamon and bay leaves, is a class of volatile phenylpropenes with characteristic aroma and comprises extensive groups of diverse amphipathic phenolic compounds, exhibiting excellent antifungal activity (Koeduka et al., 2013; Li & McClements, 2014; Roberts, McAinsh, Cantopher, & Sandison, 2014). The principal antibacterial mechanism of Eu is due to its increasing non-specific permeability of cytoplasmic membrane so as to result in the disruption of the membrane, and the suppression of enzyme action caused by hydroxyl group on Eu getting bind to proteins.

Despite its potential application and demand in food field, the efficiency of Eu is substantially limited for its high volatility, low water-solubility and highly susceptibility to environmental, processing and/or gastrointestinal conditions. These deficiencies can be effectively alleviated by the utilization of encapsulated compounds instead of free ones (Li, Hu, Du, & McClements, 2011). Available encapsulation systems include emulsions (McClements & Li, 2010), liposomes (Imran et al., 2012), solid lipid nanoparticles

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(Woranuch & Yoksan, 2013) and so on. Among those, emulsions, able to assure a uniform distribution of partially or entirely hydrophobic compounds in a hydrophilic matrix, have been the most popular used systems in commerce (Terjung, Loffler, Gibis, Hinrichs, & Weiss, 2012). Nanoemulsions (radius < 100 nm) exhibit much better stability to gravitational separation and aggregation than conventional emulsions (radius > 100 nm) attributed to the smaller droplet size (McClements, 2010). On the other hand, it is of great significance to develop a better understanding in the basic research of establishing stable emulsions with long-term storage stability for its belonging to thermodynamically unfavorable systems. It is worthy to note that nanoemulsions based on essential oils are known to be unstable against ripening or coalescence, due to their relative high water-solubility, low molecular weight and high movability (Chang, McLandsborough, & McClements, 2012; Chang, McLandsborough, & McClements, 2015; McClements, Henson, Popplewell, Decker, & Choi, 2012).

The instability of essential oil-based nanoemulsions is mainly caused by Ostwald ripening, which is attributed to diffusion of the dispersed phase through the continuous phase and a spontaneous trend toward a minimal interfacial area between the continuous phase and the dispersed one (Nazarzadeh, Anthonypillai, & Sajjadi, 2013). Therefore, ostwald ripening will cause the growth of larger particles at the expense of smaller ones with the dispersed phase being transported through the continuous phase over time. The common strategy to prevent this ripening is by incorporating another organic phase to decrease the water-solubility of the organic phase. Another organic phase, usually assigned as ripening inhibitor, is a high hydrophobic substance added to lipid droplets to slow down or retard ostwald ripening, such as long-chain triglycerides and medium-chain triglycerides. Chang et al. found that Ostwald ripening of thyme oil emulsions could inhibited with corn oil ≥60 wt% or MCT ≥50 wt% in the mixed oil phase (Chang et al., 2012). Liang et al. prepared stable peppermint oil nanoemulsions after adding MCT into the oil phase (Liang et al., 2012).

However, the phenomenon under different surfactants is rarely reported and we found that surfactants had apparent influence on the stability of Eu emulsions. In the present study, Eu emulsions were prepared by high-energy emulsification methods. Sodium dodecylsulfate (SDS) and Tween 80 (T80) were chosen as the typical ionic and nonionic surfactants to stabilize the Eu emulsions. BO was used to be the ripening inhibitor. The occurrence and suppression of ripening phenomenon under different surfactants were investigated through measuring the particle size and particle size distribution of emulsion droplets by dynamic light scattering. Further, the effect of emulsion encapsulation and composition modification on antibacterial activity of Eu was evaluated.

2. Materials and methods

2.1. Materials

Eugenol (Eu, >99% GR assay) was supplied by Aladdin Reagent Database Co. (Shanghai, China). Bean oil (BO) was purchased from a local supermarket without further purification. Tween 80 (T80) and sodium dodecylsulfate (SDS) were from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Agar were supplied from Biosharp, Japan. Mueller Hinton broth (MHB) was purchased from Qingdao Hope Biol-Technology Co., Ltd (Qingdao, China). Distilled water with a resistance of 18.2 MΩ cm was used to prepare all aqueous solutions. *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) were obtained from culture collection in Department of Food Science and Technology, Huazhong Agricultural University (Wuhan, China).

2.2. Emulsion preparation

Emulsions with a lipid phase of 5 wt% and an aqueous phase of 95 wt% were prepared by high-pressure homogenizer. Lipid phase was prepared by mixing different mass ratios of Eu and BO prior to homogenization. The aqueous phase consisted of 1 wt% T80 or SDS was prepared by dissolving T80 or SDS powder in deionized water. Coarse pre-emulsions were obtained by blending lipid and aqueous phases with a Polytron homogenizer at 26,000 rpm for 3 min at ice bath, and then the resulting pre-emulsions were homogenized by passing through a high pressure microfluidizer (Microfluidics M-110L, Microfluidics Corp., Newton, MA, USA) at 11,000 psi for five passes. After preparation, the nanoemulsions were stored at 4 °C prior to analysis. Eu-SDS and Eu-T80 meant the emulsions stabilized by SDS and T80, respectively. BO-T80 meant the emulsions stabilized by T80 and with 100% BO as the oil phase.

2.3. Particle size measurement

Particle size of nanoemulsion droplets were measured using a dynamic light scattering instrument (Zetasizer Nano ZS, model ZEN 3600, Malvern Instrument, Malvern, U.K.) at a scattering angle of 173° with each measurement being an average of 15 runs. The light source was a laser beam operating at 658 nm. The nanoemulsions were diluted in deionized water until the droplet concentration was approximately 0.005% (w/v) prior to measurement to prevent multiple scattering effects. The particle size data were reported as the intensity-weighed ("Z-average") mean particle diameter.

2.4. Microbial cultures

Two strains of foodborne gram-negative bacteria *Escherichia coli* (*E. coli*) and gram-positive pathogenic bacteria *Staphylococcus aureus* (*S. aureus*) were selected as the representative microorganisms. The Stock cultures were frozen at −20 °C in glycerol. Working cultures were obtained by transferring 1 mL stock cultures into 100 mL sterile MHB, grown at 37 °C for 24 h to 10^7 CFU/mL and then diluted into 10^4 CFU/mL before test.

2.5. Determination of minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs)

96-well plate microdilution method was used to determine MICs and MBCs of Eu nanoemulsions according to the previous reports with slight modification (Rex, 2008; Perumal, Pillai, Cai, Mahmud, Ramanathan, 2012). Briefly, an identical two-fold serial dilution of Eu nanoemulsions, ranging from 20,000 μg/mL-156.25 μg/mL, was prepared by sterile MHB in 96-well plate. Then 100 μL bacterial inoculum was added in all wells and mixed thoroughly to give final concentrations ranging from 10,000 μg/mL-78.12 μg/mL. The cultured 96-well plates were sealed with parafilm and incubated at 37 °C for 24 h. Then 50 μL of 0.2 mg/mL p-iodonitrotetrazolium chloride (INT, Sigma–Aldrich, USA) was added in all wells, and subsequently incubated at 37 °C for 30 min. MIC is defined as the lowest concentration showing no color change (clear) and exhibiting complete inhibition of bacterial growth (Perumal et al., 2012). Then, 100 μL solutions collected from each well were inoculated onto Mueller-Hinton agar (MHA) to determine MBC. The number of surviving organisms was counted after over-night incubation at 37 °C. The lowest concentration where less than 0.1% of the initial inoculum survived was deemed to MBC (Hammer, Carson, & Riley, 1996). Experiments of each sample were carried out in triplicates. BO-T80 nanoemulsion (free of Eu) was acted as the positive control while the negative control was sterile normal saline without bacterial inoculum. The MIC and MBC of bulk