Food Chemistry 206 (2016) 167–173

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Nanoemulsions of thymol and eugenol co-emulsified by lauric arginate and lecithin

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ARTICLE INFO

Article history:
Received 31 October 2015
Received in revised form 15 March 2016
Accepted 18 March 2016
Available online 19 March 2016

Keywords:
Lauric arginate
Lecithin
Nanoemulsion
Essential oil
Antimicrobial properties

ABSTRACT

Lauric arginate (LAE) is a cationic surfactant with excellent antimicrobial activities. To incorporate essential oil components (EOCs) in aqueous systems, properties of EOC nanoemulsions prepared with a LAE and lecithin mixture were studied. The LAE–lecithin mixture resulted in stable translucent nanoemulsions of thymol and eugenol with spherical droplets smaller than 100 nm, contrasting with the turbid emulsions prepared with individual emulsifiers. Zeta-potential data suggested the formation of LAE–lecithin complexes probably through hydrophobic interaction. Negligible difference was observed for antimicrobial activities of nanoemulsions and LAE in tryptic soy broth. In 2% reduced fat milk, nanoemulsions showed similar antilisterial activities compared to free LAE in inhibiting *Listeria monocytogenes*, but was less effective against *Escherichia coli* O157:H7 than free LAE, which was correlated with the availability of LAE as observed in release kinetics. Therefore, mixing LAE with lecithin improved the physical properties of EOC nanoemulsions but did not improve antimicrobial activities, especially against Gram-negative bacteria.

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1. Introduction

Lauric arginate (LAE; ethyl-N$^{\alpha}$-lauroyl-L-arginine ethyl ester monohydrochloride) is a cationic antimicrobial derived from lauric acid, arginine and ethanol (Ruckman, Rocabayera, Borzelleca, & Sandusky, 2004). LAE has been approved as a generally recognized as safe (GRAS) preservative by the United States Food and Drug Administration (USDA, 2005). LAE has very low toxicity because it is rapidly metabolized *in vivo* to lauric acid and arginine, both of which are naturally occurring dietary components (Hawkins, Rocabayera, Ruckman, Segret, & Shaw, 2009). These features make LAE a promising antimicrobial preservative to control foodborne pathogens in food systems. It inhibits a broad spectrum of foodborne pathogens (Ma, Davidson, & Zhong, 2013; Ma, Zhang, & Zhong, 2016; Porto-Fett et al., 2010) and, to date, LAE has been reported in many studies to be a highly efficient antimicrobial agent (Higueras, López-Carballo, Hernández-Muñoz, Gavara, & Rollini, 2013; Noll, Prichard, Khaykin, Sinko, & Chikindas, 2012; Saini, Barrios, Marsden, Getty, & Fung, 2013). In a recent study in our laboratories, the minimum inhibitory concentration (MIC) of LAE for inhibiting *Listeria monocytogenes* Scott A was found to be 11.8 ppm in tryptic soy broth (TSB), while the MIC for *Escherichia coli* O157:H7 ATCC 43895 or *Salmonella* Enteritidis was 23.5 ppm (Ma, Davidson, & Zhong, 2013).

One problem with LAE is that, as a cationic antimicrobial, its antimicrobial activity is reduced considerably when applied in complex food matrices (Ma et al., 2013) due to binding with food components, such as anionic biopolymers (Asker, Weiss, & McClements, 2008; Bonnaud, Weiss, & McClements, 2010). For example, even at 750 ppm, LAE did not completely inhibit 6 log CFU/mL of *E. coli* O157:H7 ATCC 43895 or *S.* Enteritidis in 2% reduced fat milk after incubation at 21 °C for 48 h (Ma et al., 2013). Additionally, the cationic nature of LAE causes a bitter taste at high concentrations (Zheng, 2014), which affects the acceptability of food products. Thus, strategies are needed to improve the functionality of LAE.

Some spice essential oils (EOs) or essential oil components (EOCs) have strong antimicrobial activity (Burt, 2004; Ma et al., 2016; Zhang, Ma, Critzer, Davidson, & Zhong, 2015) and are promising natural antimicrobial preservatives. Like LAE, binding by proteins and lipids requires high concentrations of EOs/EOCs to obtain sufficient inhibition of foodborne pathogens in complex food matrices such as milk (Chen, Davidson, & Zhong, 2014; Ma et al., 2013). EOs/EOCs can also affect the sensory aspects and acceptability of food products (Busatta et al., 2008; Nielsen &

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http://dx.doi.org/10.1016/j.foodchem.2016.03.065
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Rios, 2000). Therefore, approaches for lowering the usage level of EOs/EOCs in foods are needed.

Preservation using antimicrobial combinations is an effective way to lower the concentration of each antimicrobial if synergistic antimicrobial effectiveness can be obtained. In our recent study, combining LAE and EO/EOC (eugenol, thymol, and cinnamon leaf oil) pre-dissolved in ethanol showed a synergistic antimicrobial effect against *L. monocytogenes* Scott A (Ma et al., 2013). Since EOs/EOCs are hydrophobic and have limited solubility in water (Chen et al., 2014), colloidal systems, such as oil-in-water nanoemulsions, are needed to incorporate EOs/EOCs in aqueous systems (Chang, McLandsborough, & McClements, 2015; Guan, Wu, & Zhong, 2016; Moghimi, Ghaderi, Rafati, Aliahmadi, & McClements, 2016). Because LAE is also an emulsifier, it can be used to prepare EO/EOC nanoemulsions (Ziani, Chang, McLandsborough, & McClements, 2011). To reduce the level of LAE as an emulsifier, another GRAS emulsifier may be used to co-emulsify EOs/EOCs. In recent studies, we have observed synergistic surface activity when hydrophobic lecithin was used in combination with water-soluble sodium caseinate, gelatin, or Tween 20 to prepare nanoemulsions or microemulsions of EOs/EOCs (Chen, Guan, & Zhong, 2015; Xue & Zhong, 2014a, 2014b). Therefore, the objective of the present study was to prepare and characterize emulsions of eugenol or thymol using a combination of LAE and lecithin. Physical properties were studied for dimension, storage stability, zeta-potential, and morphology of emulsion droplets, as well as release kinetics of LAE. Antimicrobial activities of emulsions were characterized in TSB and 2% reduced fat milk using a Gram-positive bacterium, *L. monocytogenes* Scott A, and two Gram-negative bacteria, *E. coli* O157:H7 ATCC43895 and *S.* Enteritidis.

2. Materials and methods

2.1. Materials

LAE was provided by Vedeqsa Inc. (New York, NY). The commercial product Mirenat-TT contained 15.5% w/w LAE, with other components being propylene glycol and polysorbate. Eugenol (98% purity) and thymol (⩾99% purity) were purchased from Sigma–Aldrich Corp. (St. Louis, MO). Soy lecithin (major component being phosphatidylcholine) was from Thermo Fisher Scientific Inc. (Waltham, MA). Chemicals were used without further purification. Simple Truth® 2% ultra-pasteurized reduced fat milk with a fat content of 2.08% w/v and a protein content of 3.33% w/v (Kroger Co., Cincinnati, OH) was bought from a local store.

2.2. Bacterial culture

L. monocytogenes Scott A, *E. coli* O157:H7 ATCC43895, and *S.* Enteritidis were from the culture collection of Department of Food Science and Technology at the University of Tennessee in Knoxville. All strains were stored in sterile 20% glycerol at −20 °C and transferred at least 2 times in TSB with an interval of 24 h before use. *L. monocytogenes* was incubated at 32 °C, while *E. coli* O157:H7 and *S.* Enteritidis were incubated at 37 °C.

2.3. Preparation of nanoemulsions

Lecithin was mixed at 1% w/w in deionized (DI) water, followed by adding 3–7% w/w Mirenat-TT (corresponding to 0.47–1.09% w/w LAE) and 1% w/w eugenol. The mixture was then homogenized at 15,000 rpm for 6 min using a T25 digital UlTRA TURRAX® homogenizer (IKA® Works, Inc., Wilmington, NC). Absorbance at 600 nm of emulsions was measured using a UV–Vis spectrophotometer (Evolution 201, Thermo Scientific, Waltham, MA). The optimized conditions identified for eugenol were then used to prepare the nanoemulsion of thymol. Treatments with both LAE and lecithin were prepared in triplicate, while those with LAE only were prepared in duplicate.

2.4. Dimension and stability of emulsion droplets

The hydrodynamic diameter of nanoemulsions was measured using a model Delsa™ Nano C particle size/zeta-potential analyzer (Beckman Coulter, Atlanta, GA) during 30-day storage at room temperature (21 °C). Samples were diluted in deionized (DI) water before measurement. Three nanoemulsion replicates were studied.

2.5. Atomic force microscopy (AFM)

The morphology of nanoemulsion droplets was studied using AFM. Nanoemulsions were diluted 1000 times in DI water. Ten microliter of the diluted sample was spread on a freshly cleaved mica sheet and mounted on a sample holder (Bruker Corp., Santa Barbara, CA). After about 2-h drying, samples were scanned in the tapping mode with a Multimode VIII microscope (Bruker AXS, Billerica, MA, USA). Topography images scanned at a dimension of 1.0 × 1.0 μm were collected.

2.6. Zeta-potential measurement

The zeta-potential of LAE, lecithin, LAE and lecithin mixture, and eugenol nanoemulsions prepared with LAE and lecithin were measured at 25 °C (model Nano-ZS Zetasizer, Malvern Instruments Ltd, Worcestershire, UK). Nanoemulsions were diluted in DI water and adjusted to pH 4.0–7.0 using 1.0 M HCl or NaOH before measurement. Three measurements with 3 runs each were done for each sample.

2.7. Release kinetics of LAE

Release kinetics of LAE from nanoemulsions was studied by dialysis against DI water at room temperature (21 °C). Regenerated cellulose dialysis tubing with a molecular weight cut-off of 3500 Da (Thermo Fisher Scientific Inc., Waltham, MA) was loaded with 5 mL nanoemulsions or a 6000 ppm LAE solution that was identical to the LAE concentration of the nanoemulsion. The sealed tubes were placed in beakers containing 200 mL DI water that was mixed on a stir plate at 300 rpm. 20 mL of solution outside the dialysis tubing was withdrawn after 0, 1, 2, 4, 8, 24, 48, 72, 96 h, and 20 mL of fresh DI water was added to the beakers to maintain the volume at each sampling. LAE concentration in the sample withdrawn was quantified with HPLC (Higueras et al., 2013). Briefly, the reversed-phase HPLC system (Agilent 1200 series; Agilent Technologies, Waldbronn, Germany) was equipped with a UV detector (204.16 nm). A Zorbax Eclipse Plus C18 HPLC column (4.6 × 150 mm, 5 μm; Agilent, Palo Alto, CA) protected by a Zorbax Eclipse Plus C18 guard column (4.6 × 12.5 mm, 5 μm) was used. The sample injection volume was 10 μL and the mobile phase with equal volumes of acetonitrile and water acidified with 0.1% trifluoroacetic acid was run at 1.0 mL/min. The cumulatively released LAE was calculated using the following equation (Xiao & Zhong, 2011):

$$R_{t_i}\ (\%) = \frac{\sum_{n=1}^{i-1} a_n \times 20 + a_i \times 200}{A \times 5} \times 100\%$$

where R_{t_i} is the cumulatively released LAE at time t_i, a_i is the concentration of LAE outside the dialysis tube at time t_i, and A is the original concentration of LAE in the dialysis tube. All experiments were repeated in triplicate.

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