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Physical and antimicrobial properties of cinnamon bark oil co-nanoemulsified by lauric arginate and Tween 80

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ABSTRACT

Lauric arginate (LAE) is a water-soluble cationic surfactant which has antimicrobial activity against a broad spectrum of foodborne pathogens. Some spice essential oils are effective lipophilic antimicrobials. Combining both antimicrobials may reduce their usage levels and possible negative sensory impacts when applied in complex food matrices. The objective of this study was to combine a nonionic surfactant (Tween 80) with LAE to form stable nanoemulsions with cinnamon bark essential oil (CBO) and to characterize the antimicrobial activity of these nanoemulsions. CBO was homogenized at 1% w/w in the aqueous phase with 3% w/w Tween 80 and 0.05–0.375% w/w LAE, followed by heating at 90 °C for 30 min to obtain final emulsions. With 0.125% and higher LAE, transparent emulsions with ~100 nm in hydrodynamic diameter were observed to be stable during 30-day storage at 21 °C. Antimicrobial activities of the nanoemulsion prepared with Tween 80 and 0.375% w/w LAE were studied. The respective minimum inhibitory concentrations (MICs) of the nanoemulsion in tryptic soy broth (TSB) were 12, 7, and 8 ppm LAE for *Salmonella enteritidis*, *Escherichia coli* O157:H7, and *Listeria monocytogenes*, while those of free LAE were 11, 6, and 6 ppm, respectively. MICs of CBO were 400 ppm for the tested bacteria and Tween 80 at 6% w/w did not show inhibitory effect. Growth kinetics of the bacteria in TSB treated with the nanoemulsion or individual components at concentrations corresponding to the MICs of free LAE showed that binding among the LAE and Tween 80 and CBO components resulted in the antibacterial activity of nanoemulsion being lower than same concentrations of free LAE and CBO. Conversely, little difference was observed for the individual antimicrobials and the nanoemulsion in 2% reduced fat milk, and 750 ppm LAE and 2000 ppm CBO were observed to be the dominant antimicrobial against Gram-positive and Gram-negative bacteria, respectively. The growth of *L. monocytogenes* in 2% reduced fat milk at 4 °C was not observed when treated by the nanoemulsion corresponding to 187.5 ppm LAE and 500 ppm CBO. Therefore, stable and transparent nanoemulsions of EOs can be prepared with the combination of LAE and Tween 80 without compromising antimicrobial activities.

1. Introduction

A big challenge for the food industry is to ensure the microbiological safety and quality of food products. Essential oils (EOs) are volatile and aromatic oily liquids obtained from plants such as thyme, basil, oregano and cinnamon (Oussalah et al., 2007). They are gaining interest for their potential use as preservatives because they are generally recognized as safe (GRAS), have activities against various foodborne spoilage and pathogenic microorganisms, and are widely accepted by consumers (Burt, 2004; Elgayyar et al., 2001). Emulsions are used to incorporate EOs in aqueous food matrices, and nanoemulsions, with droplets smaller than 100 nm in diameter, are needed in applications requiring optical transparency. The small dimension of droplets can also reduce destabilization mechanisms such as gravitational separation and aggregation.

Nanoemulsions can be prepared by high energy or low energy methods, with the former applying intense mechanical energy to break up droplets in unit operations, such as high pressure or high shear homogenization, and microfluidization (Rao and McClements, 2010; Solè et al., 2006). To overcome some of the drawbacks of the high energy methods, low energy approaches such as the phase inversion temperature (PIT) method have been studied (Forgiarini et al., 2001). The PIT method is usually based on temperature-dependent molecular geometry of nonionic surfactants (Shinoda and Arai, 1964). When heated to above the PIT, surfactants with good affinity for water and oil mixtures form bicontinuous or lamellar structures because of the ultralow interfacial tension (Rao and McClements, 2010). After rapid cooling through the PIT with continuous stirring, stable nanoemulsions can be formed as a result of redistribution of surfactants between water and oil phases (Roger et al., 2010). The coalescence of

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droplets after nanoemulsion formation is one of the major problems associated with the PIT method (Izquierdo et al., 2002). To improve the kinetic stability of nanoemulsions formed by the PIT method, a nonionic surfactant, polysorbate 80 (Tween 80), or an anionic surfactant, sodium dodecyl sulfate (SDS), was used in combination with polyoxyethylene-(4)-lauryl ether (Brij® 30) to change the optimum curvature of the oil/water interface and to increase repulsive colloidal interactions between the droplets (Rao and McClements, 2010). However, droplet growth due to Ostwald ripening still can occur because EO components have some water-solubility (Chen et al., 2014). Physically, Ostwald ripening effect is due to the higher solubility of lipophilic compounds in smaller particles, which results in molecules dissolving out of smaller droplets to join bigger droplets, causing the gradual reduction and eventual disappearance of smaller droplets and the continual growth of bigger droplets (Capek, 2004).

Although nanoemulsions have been reported to enhance antimicrobial activity of EOs by increasing their solubility in water (Donsì et al., 2012), opposite trends have also been observed. For example, the activity against *Listeria innocua* was lower after carvocrol was emulsified by Tween 80 as smaller droplets (Terjung et al., 2012). It was determined that the concentration of carvacrol in the aqueous phase decreased with a decrease in the droplet dimension and carvacrol was preferentially located at the oil-water interface. The binding between EOs and surfactants used in emulsification therefore may reduce the availability of EOs and subsequently antimicrobial activity.

It is also known that EOs used as antimicrobials have strong flavors which are often not compatible with foods for which they might be applied. Increasing activity is one method for overcoming that problem and the other is to use other antimicrobials in combination with EOs to reduce their concentration required to inhibit microbial growth. For this reason, lauric arginate (N^{α}-lauroyl-L-arginine ethyl ester monohydrochloride, LAE) is particularly interesting because it is a cationic surfactant and is GRAS for use as an antimicrobial preservative in certain food applications (Bakal and Diaz, 2005; USDA, 2005). Recently, synergistic antimicrobial activity against *Listeria monocytogenes* was observed for the combinations of LAE and cinnamon leaf oil or eugenol, while the activity was additive for the combination of LAE and thymol (Ma et al., 2013). Conversely, an antagonistic effect was found for the same combinations against *Escherichia coli* O157:H7 and *Salmonella enteritidis*.

Utilization of LAE as an antimicrobial is not without its challenges. Anionic compounds in food matrices, such as proteins in milk, can bind with cationic LAE and greatly increase the concentration required to achieve similar inhibitions in microbial growth media, such as tryptic soy broth (TSB) (Ma et al., 2013). Binding between LAE and anionic biopolymers (mucin) in the mouth also leads to a bitter taste (Bonnaud et al., 2010). LAE was observed to form mixed micelles with nonionic polyethylene glycol sorbitan monolaurate (Tween 20), and the reduction of zeta-potential from +37 to +6 mV was found to potentially reduce the bitter taste of LAE (Asker et al., 2011). Recently, a combination of 0.1% LAE and 0.9% Tween 80 was demonstrated to be more feasible than 1% Tween 80 in forming stable turbid nanoemulsions with 10% oil phase with different mass ratios of thyme oil and corn oil by high pressure homogenization (Chang et al., 2015). Corn oil effectively inhibited Ostwald ripening of EO emulsions but drastically reduced antimicrobial activity of EOs (Chang et al., 2012).

Therefore, much work is needed to study EO nanoemulsions prepared with the combination of LAE and nonionic surfactants that could utilize the activities of both antimicrobials to reduce their usage levels to eventually improve sensory characteristics. The objective of the present work was to study physical and antimicrobial properties of emulsions prepared with non-ionic surfactants Tween 20 and Tween 80 and different concentrations of LAE. The PIT method was used for nanoemulsion formation. Cinnamon bark oil (CBO) was chosen as a model EO because it is one of the most effective EOs against common foodborne pathogens (Cava-Roda et al., 2012; Chang et al., 2001). Cinnamaldehyde is the major component (50–80%) of CBO, while other minor constituents include eugenol (10%), safrole (0–11%), linalool (10–15%), and camphor (Jian Qin, 2003). The antimicrobial properties of nanoemulsions and the same concentrations of free antimicrobials were compared in TSB and 2% reduced fat milk. Milk is used as a model food matrix because anionic proteins and hydrophobic fat globules can interfere with antimicrobial activities of cationic LAE and lipophilic EOs (Ma et al., 2013; Chen et al., 2014). This information can then be used to guide the application of nanoemulsions in specific food matrices.

2. Materials and methods

2.1. Chemicals

LAE was purchased from Vedeqsa Inc. (New York, NY). The commercial product with a name of Mirenat-TT contained 15 ± 0.5% w/w LAE. Tween 20 and Tween 80 were purchased from Acros Organics (Morris Plains, NJ). TSB, agar and yeast extract were purchased from Thermo Fisher Scientific Inc. (Pittsburgh, PA). The ultra-pasteurized 2% reduced fat milk (Simple Truth Organic, San Diego, CA) was purchased from a local grocery store. CBO was purchased from Sigma-Aldrich Corp. (St. Louis, MO).

2.2. Emulsion preparation

CBO was mixed at 1% w/w with the aqueous phase containing 3% w/w Tween 80 or Tween 20 and 0–0.375% w/w LAE. The mixtures were homogenized at 15,000 rpm for 4 min (model Ultra Turrax, IKA Works Inc., Wilmington, NC). These emulsions were also heated at 90 °C for 30 min, followed by quenching in ice/water (Forgiarini et al., 2001).

2.3. Droplet size and turbidity of emulsions

The hydrodynamic diameter (d_h) of emulsion droplets was measured with a DelsaNano C particle analyzer from Beckman Coulter Inc. (Brea, CA). The emulsion consisting of 0.05% w/w LAE was diluted in deionized water at a mass ratio of 1:10, while this mass ratio was 1:5 for other samples to meet the instrument sensitivity requirement. In addition to d_h, polydispersity indexes (PDIs) of emulsions were reported because PDI indicates the heterogeneity of droplet dimension and an increase of PDI indicates coalescence and/or Ostwald ripening in emulsions (McClements, 2004). The turbidity of emulsions was measured without dilution for absorbance at 600 nm using a BioMate 5 UV–Vis spectrophotometer from Thermo Fisher Scientific Inc. (Pittsburgh, PA).

2.4. Bacterial culture

One Gram-positive bacterium, *L. monocytogenes* Scott A, and two Gram-negative bacteria, *E. coli* O157:H7 ATCC 43895, and *S. enteritidis*, were obtained from the culture collection of the Department of Food Science and Technology at the University of Tennessee in Knoxville. All strains were maintained at −20 °C in 20% glycerol. Each strain was transferred at least 2 times in TSB with an interval of 24 h before use. Except growth kinetics experiments, *L. monocytogenes* was incubated at 32 °C while *E. coli* O157:H7 and *S. enteritidis* were incubated at 37 °C.

2.5. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC was determined using the microbroth dilution method (Branen and Davidson, 2004). The bacterial culture was diluted to about 10^6 CFU/mL in TSB. 120 μL of the diluted culture was added into wells of a 96-well microtiter plate. Antimicrobial stock solution was diluted in TSB in series from 10 to 40 ppm LAE. Free CBO samples were prepared by dissolving at 10% w/*v* in ethanol, followed by dilution in