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Development and characterization of geraniol-loaded polymeric nanoparticles with antimicrobial activity against foodborne bacterial pathogens

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ABSTRACT

This study demonstrates the rose essential oil component (EOC) geraniol can be loaded into polymeric nanoparticles (NPs) with sustainable release profile. Geraniol-loaded NPs were prepared by flash nanoprecipitation and characterized for size, encapsulation efficiency, payload release during storage, inhibition of *Escherichia coli* O157:H7 and *Salmonella enterica* Typhimurium *in vitro* and on spinach surfaces, and NP-assisted transport of EOC into cellular membranes. Adjusting concentrations of stabilizing polymer, Pluronic® F-127, and geraniol produced NPs ranging in size from 26 to 412 nm. Antimicrobial NPs inhibited *S.* Typhimurium and *E. coli* O157:H7 growth at 0.25 and 0.2 wt.%, respectively. Geraniol-loaded NPs displayed sustained release with a time constant of 24 h, maintaining their antipathogenic properties over a prolonged time period. Pathogen reductions on treated spinach surfaces ranged from 0.3 to 4.2 $\log_{10}$ CFU/cm^2. Antimicrobial NPs may be useful for post-harvest decontamination of foods such as fresh produce from cross-contaminating microbial pathogens.

1. Introduction

Foodborne microbial pathogens continue to impose significant public health and financial burden in the U.S. despite the advent of numerous food processing technologies and food safety oversight systems designed to prevent pathogen transmission to consumers. According to the U.S. Centers for Disease Control and Prevention (CDC; Atlanta, GA), approximately 48 million incident cases of human foodborne disease occur annually, resulting in an estimated cost to the U.S. economy of 77.7 billion USD (Scallan et al., 2011a, 2011b; Scharff, 2012). Bacterial pathogens such as *Salmonella enterica* and the Shiga toxin-producing *Escherichia coli* (STEC), including *E. coli* O157:H7, have been transmitted to consumers by a variety of food vehicles, including fresh and minimally processed produce, dairy products, meat and poultry (Painter et al., 2013).

In recent years a great deal of research has been completed detailing the efficacy of plant-derived essential oil components (EOC) (e.g., thymol, carvacrol, allicin, geraniol, limonene, etc.) to inhibit the growth of foodborne bacterial pathogens (Davidson et al., 2013). Nevertheless, their utility in foods may be limited by their impacts on organoleptic properties of the food at levels sufficient to inhibit microbial growth or by low solubility in the aqueous phase of various foods (Burt, 2004; Weiss et al., 2009). One functional solution to these limitations is that of encapsulation within food-grade encapsulating materials (e.g., lipid, polymers) (Hill et al., 2013a; Sanguansri and Augustin, 2006). Gaysinsky et al. (2005a, b) reported entrapment of eugenol and carvacrol within

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surfactant micelles resulted in decreased growth in a liquid medium over 24 h at pH 7.0 of *E. coli* O157:H7 and *Listeria monocytogenes* when compared to non-encapsulated EOCs. Gomes et al. (2011, 2008), likewise, reported inhibition of bacterial pathogens on spinach surfaces treated with β-cyclodextrin-entrapped cinnamon and clove oils, reporting reduced minimum inhibitory concentrations (MIC) of encapsulated antimicrobial oils versus non-encapsulated EOC.

The rose oil component geraniol (*trans*-3,7-Dimethyl-2,6-octadien-1-ol) has been previously reported to exert growth-inhibiting activity against various foodborne bacterial and fungal microbes (Pattnaik et al., 1997; Singh et al., 2012). Kim et al. (1995) reported minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of the compound in 1% Tween 20 against *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Typhimurium, and *Vibrio vulnificus* ranged between 500 and 1000 μg/ml. Friedman et al. (2004) reported bactericidal activity of geraniol against *Salmonella* and *E. coli* O157:H7 was observed rapidly at 21 °C, achieving 50% lethality of inoculated *E. coli* O157:H7 and *Salmonella* cells at levels of 0.089 and 0.031%, respectively, in clarified apple juice. An MIC for geraniol of 0.05% against *E. coli* O157:H7 grown in medium adjusted to pH 4.5 was reported, though researchers observed that at pH 7.2 the MIC of geraniol against the pathogen was >0.1% (Knight and McKellar, 2007). The objective of this study was to develop antimicrobial nanoparticles (NPs) loaded with geraniol using the triblock copolymer Pluronic® F-127 and characterize resulting physico-chemical and antimicrobial properties. Pluronic® F-127 was selected because it is inexpensive, non-toxic, bears high biocompatibility, and has been repeatedly utilized in drug delivery systems (Kabanov et al., 2002; Pham Trong et al., 2008; Shi et al., 2015; Tilley et al., 2013). Resulting NPs were characterized for size as a function of geraniol:polymer blending ratios, encapsulation efficiency, drug release profile, and antimicrobial capacity of geraniol NPs against *Salmonella* Typhimurium and *E. coli* O157:H7.

2. Materials and methods

2.1. Materials

Geraniol (>96.0%; CAS# 106-24-1) (TCI America, Portland, OR), Pluronic® F-127 (PF127; CAS# 9003-11-6) (Sigma–Aldrich Co., St. Louis, MO), and tetrahydrofuran (THF; CAS# 109-99-9; Sigma–Aldrich Co.) were purchased and used as received for the preparation of EOC-loaded NPs. Nile Red (CAS# 7385-67-3) (Tokyo Kasei Kogyo Co., LTD, Tokyo, Japan) and poly-L-lysine (CAS# 25988-63-0) (Sigma–Aldrich Co.) were used for experiments determining nanoparticle adsorption to bacterial pathogen surfaces.

2.2. Preparation of geraniol-loaded nanoparticles

Geraniol-loaded polymeric NPs were prepared with a rapid nano-precipitation method previously reported (Akbulut et al., 2009; Zhang and Akbulut, 2011). Briefly, geraniol and the amphiphilic triblock copolymer PF127 were dissolved in THF to differing ratios to determine impact of blending ratios on resulting geraniol-containing NP size. Then, THF solution was rapidly impinged against milli-Q water to produce polymer-encapsulated geraniol-bearing NPs. The flow rate of water was 50.0 mL/min, and the flow rate of the THF solution was 5.0 mL/min. Following impingement processing, the NP-contained solution was placed under a fume hood for 7.0 h to remove THF (Fig. 1).

2.3. Characterization of nanoparticles

Particle size distribution of polymeric NPs was measured by dynamic light scattering (DLS) following four-fold dilution in milli-Q water using a Zetasizer ZS90 particle size and zeta potential analyzer (Malvern Instruments, Ltd., Westborough, MA). The measurements were carried out at a scattering angle of 90° at 25 °C. Size and shape of individual NPs was characterized by use of transmission electron microscope (TEM; JEM-2010, Jeol USA, Inc., Peabody, MA) at the Microscopy Imaging Center at Texas A&M University (College Station, TX). Nanoparticle-containing solution was diluted four-fold in milli-Q water prior to drop-wise application of NP solution onto a copper grid (400 mesh) with carbon film (CF400-Cu, Electron Microscopy Sciences, Hatfield, PA). The NP sample was dried at ambient temperature prior to TEM analysis. Observations were performed at 200 kV accelerating voltage, $<2.5 \times 10^{-5}$ Pa, at ambient temperature (~25 °C).

2.4. Release kinetics of geraniol from polymeric NPs

Geraniol-containing NPs (9.0 mL) were added into standard regenerated cellulose membranes (molecular weight cut-off 12,000–14,000 Da; approximately 2.0 nm diameter cut-off) (Spectrum Laboratories, Inc., Rancho Dominguez, CA); membranes were then placed into beakers containing 200.0 mL milli-Q water. Unencapsulated geraniol was expected to passively diffuse through dialysis membranes, while NPs were expected to be unable to diffuse through membranes, preventing entrapped geraniol from diffusion. Changes in concentration of free geraniol were tracked by spectroscopy using a UV-1800 UV/Visible scanning spectrophotometer (Shimadzu Corp., Columbia, MD), scanning from 190 to 800 nm (geraniol maximal absorption: 240 nm). UV measurements were performed at ambient temperature at 1, 2, 4, 8, 24, 48, 96, and 144 h. Four independent replications were completed for each measurement.

2.5. Encapsulation efficiency (EE) of nanoparticles

Geraniol loading and EE was determined by mass spectrometry (MS). Nanoparticles were added to hexane at 1:10 NP:hexane (calculated as weight NP:weight hexane) to degrade NPs and release geraniol into the solvent phase. Following geraniol release from NPs, hexane was used to complete MS analysis of geraniol at the Laboratory for Biological Mass Spectrometry (Department of Chemistry, Texas A&M University). A reference/standard solution of 0.08 wt. % geraniol was prepared to calibrate the concentration of geraniol obtained from degraded NPs in mass spectra. Ten μl of 0.5 mg/ml of hexadecanol was added as internal standard to 50 μL of sample. 100 μL of N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) with trimethylchlorosilane (TMCS) was added into this solution. The mixture was heated at 60 °C for 1 h. The compounds of interest were detected as trimethylsilyl (TMS) derivatives using GC/MS. Ultra GC/DSQ (Thermo-Electron, Waltham, MA) was used for GC–MS. Chromatography was carried out using an Rxi-5ms column (60 m × 0.25 mm with 0.25 μm film thickness) (Restek, Bellefonte, PA). Helium was used as a carrier gas at constant flow of 1.5 mL/min. GC inlet was held at 225 °C while transfer line and ion source temperatures were held at 250 °C. An aliquot of 1.0 μL of sample was injected in splitless mode. The oven temperature was maintained at 50 °C for 5 min, then raised to 320 °C at 20 °C/min. Electron impact ionization at 70 eV was used for ionization and mass spectra were acquired in full scan mode in the range of 30–500 m/z. Three replications were completed for MS analysis of NP EE; EE was determined as: