LWT - Food Science and Technology 51 (2013) 86–93

Contents lists available at SciVerse ScienceDirect

LWT - Food Science and Technology

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

Characterization of beta-cyclodextrin inclusion complexes containing essential oils (*trans*-cinnamaldehyde, eugenol, cinnamon bark, and clove bud extracts) for antimicrobial delivery applications

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ARTICLE INFO

Article history:
Received 31 May 2012
Received in revised form
1 November 2012
Accepted 7 November 2012

Keywords:
Essential oils
Natural antimicrobials
Beta-cyclodextrin inclusion complexes
Listeria spp.
Salmonella spp

ABSTRACT

This study aimed to elucidate the physico-chemical characteristics of EO–BCD inclusion complexes and their resulting antimicrobial activity. Cinnamon bark extract, *trans*-cinnamaldehyde, clove bud extract, eugenol, and a 2:1 (*trans*-cinnamaldehyde:eugenol) mixture were microencapsulated by the freeze-drying method. EO–BCD complexes were characterized for particle size, morphology, polydispersity index, entrapment efficiency, and phase solubility. All particles showed a spherical shape, smooth surface, no significant differences in size distribution and strong tendency to agglomerate. The entrapment efficiencies ranged from 41.7 to 84.7%, where pure compounds were higher ($p < 0.05$) than extracts. The oils and their BCD complexes were analyzed for their antimicrobial activity against *Salmonella enterica* serovar Typhimurium LT2 and *Listeria innocua*. All antimicrobials effectively inhibited bacterial growth within the concentration range tested, except free eugenol. The EO–BCD complexes were able to inhibit both bacterial strains at lower active compound concentrations than free oils, likely due to their increased water solubility that led to increased contact between pathogens and essential oils. The cinnamon bark and clove bud extract BCD complexes were the most powerful antimicrobials, despite showing the lowest entrapment efficiencies amongst the oils. Results suggest that the application of these antimicrobial complexes in food systems may be effective at inhibiting pathogens.

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1. Introduction

The Centers for Disease Control and Prevention (CDC) have estimated that up to 48 million illnesses, 128,000 hospitalizations, and 3000 deaths each year in the United States are caused by foodborne pathogens (CDC, 2011, 2012b). Symptoms of the illnesses caused by these pathogens may range from mild gastroenteritis to life-threatening neurological, hepatic, or renal syndromes. *Salmonella* and *Listeria monocytogenes* are two of the major pathogens that cause foodborne illnesses. *Salmonella* are the most common bacterial pathogens associated with foodborne outbreaks around 1.2 million cases of salmonellosis occur in the United States annually. Infection often leads to diarrhea, fever, and abdominal cramps and can lead to severe illness in young children, the elderly population and anyone who is immune-compromised (CDC, 2011, 2012a). *L. monocytogenes* has been attributed to foodborne pathogen outbreaks on a variety of food products (ready-to-eat meat, poultry products, and vegetables) and can cause serious health problems including still births, premature delivery, meningitis, and septicemia (Swaminathin, Cabanes, Zhang, & Cossart, 2007). There is a need in the food industry for effective technologies to inactivate foodborne pathogens on food products and ensure their safety for consumers.

Essential oils (EOs) have been shown to be powerful natural antimicrobials against a variety of foodborne pathogens (Kim, Marshall, & Wei, 1995). Their "natural" reputation makes them highly desirable for use in food products, as consumers have become weary of chemical or synthetic additives in their foods in recent history. Many have already been granted Generally Recognized as Safe (GRAS) status by the FDA in 21 CFR (Code of Federal Regulation) part 172.515 (CFR, 2009), meaning they can be used in food products without further approval. Different EOs exhibit varying inhibitory effects on microbial pathogens. Cinnamon extract, *trans*-cinnamaldehyde, clove extract, and eugenol have been found to be some of the most effective antimicrobials against foodborne pathogens and it has been reported that spice extracts

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0023-6438/$ – see front matter Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.lwt.2012.11.011

are more powerful inhibitors than purified compounds (Gomes, Moreira, & Castell-Perez, 2011; Valero & Salmeron, 2003). The challenges with using these antimicrobials on food products are: (i) they have an extremely low flavor threshold and can drastically change the sensory properties of the food and (ii) they are highly insoluble in water due to their lipophilic nature and may have limited contact with pathogens in high moisture content foods (Kalemba & Kunicka, 2003).

One way to avoid or lessen these effects is through an encapsulation process a technique designed to improve aqueous solubility and potentially enhance delivery of antimicrobials. Cyclodextrins are enzymatically modified starch that have the ability to form inclusion complexes with hydrophobic molecules such as essential oils (Mourtzinos, Kalogeropoulos, Papadakis, Konstantinou, & Karathanos, 2008). Cyclodextrins can not only mask the flavor of essential oils to be used as antimicrobials but can also protect against oxidation or heat damage, allowing the essential oils to remain effective as antimicrobial agents under a wide variety of environmental conditions and for longer time periods (Hedges, Shieh, & Sikorski, 1995; Qi & Hedges, 1997). The type of cyclodextrin (α-, β-, γ-) indicates its size and therefore the size of the hydrophobic molecule that the cyclodextrin can entrap in its inner cavity (Karathanos, Mourtzinos, Yannakopoulou, & Andrikopoulos, 2007). The interaction between BCD (host) and active compounds (guests) may involve total inclusion or association with the hydrophobic or hydrophilic part of the molecule (Szente & Szejtli, 2004). Beta-cyclodextrin is GRAS, appropriately sized cyclodextrin for forming 1:1 inclusion complexes with *trans*-cinnamaldehyde, eugenol, cinnamon bark extract, and clove bud extract molecules (Benita, 2006; USFDA, 2001). Once oils are within the inclusion complexes, their sensory impact on food products can be reduced and their water solubility increased, providing sufficient contact with pathogens to inhibit their growth, making foods safer for human consumption. Considering the requirements of natural antimicrobial application effectiveness and convenience in food systems, the goal of this study was to determine the physico-chemical characteristics of various EO–BCD inclusion complexes and their resulting antimicrobial activity against foodborne pathogens.

2. Materials and methods

2.1. Materials

All extracts and essential oils were of food-grade quality and were purchased from Sigma Aldrich Co. (St. Louis, MO). The *trans*-cinnamaldehyde (93%), eugenol (99%), clove bud and cinnamon bark extracts (100%) were used in this study. Beta-cyclodextrin (BCD) hydrate was purchased from Alfa Aesar (Ward Hill, MA), and tryptic soy agar (TSA), tryptic soy broth (TSB), peptone, modified Oxford's medium (MOX), and tryptose phosphate broth (TPB) for bacterial growth and enumeration were purchased from Becton, Dickinson and Co. (Franklin Lakes, NJ). HPLC-grade acetonitrile was purchased from Mallinckrodt Chemical (Hazelwood, MO). All other reagents were analytical grade.

2.2. Preparation of beta-cyclodextrin inclusion complexes

The inclusion complexes were prepared via the freeze-drying method (Karathanos et al., 2007). BCD and EOs were mixed in an aqueous solution in a 1:1 molecular ratio at concentrations of 16 mmol/L each. The mixture was magnetically stirred in a sealed container for 24 h at room temperature (25 °C) to allow for complex formation and prevent loss of volatiles to the atmosphere. After mixing, the solution was frozen and lyophilized at −50 °C and 1.09 Pa in a Labconco Freeze Dryer-5 (Kansas City, MO) for approximately 48 h or until all moisture had been sublimated. The lyophilized powders were stored in sealed containers inside a desiccator at −20 °C until use. Inclusion complexes containing *trans*-cinnamaldehyde, eugenol, 2:1 *trans*-cinnamaldehyde:eugenol, cinnamon bark extract, and clove bud extract were prepared. Calculations for mixing the 1:1 molecular aqueous solutions for the cinnamon bark and clove bud extract inclusion complexes were based upon the concentrations of active compound present (*trans*-cinnamaldehyde and eugenol, respectively). The concentration of each active compound in the spice extracts was measured spectrophotometrically at 280 nm (Shimadzu UV-1601 spectrophotometer, Columbia, MA).

2.3. Oxidative Differential Scanning Calorimetry (DSC)

Oxidative DSC was used to study complex formation between EOs and BCD. Analysis was performed using a Pyris 6 Perkin Elmer instrument (Pyris 5.0 Software, Boston, MA) with a scanning rate of 90 °C/min from 25 to 120 °C, maintained at 120 °C for 1 min to ensure even sample heating, then heated to 400 °C at a rate of 10 °C/min under an oxygen atmosphere (ultra-pure air) (Mourtzinos, Salta, Yannakopoulou, Chiou, & Karathanos, 2007). The instrument was calibrated using zinc and indium metals before sample testing. Samples of free EOs or the BCD complexes containing the same quantity of EO (~2.0 mg) were weighed to the nearest 0.1 mg into 20 μL aluminum pans and sealed with one hole in their lids.

2.4. Particle size analysis and morphology

A Delsa™ Nano C Particle Analyzer (Beckman Coulter, Brea, CA) was used to measure the average size and polydispersity indices (PDI) for each type of BCD inclusion complex particle. The particles were suspended in distilled water at a 4:1 ratio for measurement in 1 cm path length plastic cuvettes at scattering angle of 165°, with a pinhole set to 20 μm, and a refractive index of 1.3328 for 120 continuous accumulation times. PDI is a measure of the uniformity of particle sizes present in the suspension. A value close to zero (<0.10) indicates little variability in size (monodisperse), whereas values > 0.10 indicate polydisperse systems (Zigoneanu, Astete, & Sabliov, 2008). The advantage of a monodisperse system is related to its ability to deliver a consistent amount of compound, as compared to a mixture of polydisperse particles, of different loading capacities (Astete & Sabliov, 2006).

Aqueous suspensions of inclusion complex particles were examined using a JEOL 1200 EX Transmission Electron Microscope (TEM) (JEOL USA Inc., Peabody, MA) at the Microscopy Imaging Center of Texas A&M University (College Station, TX). Aqueous suspensions of particles were placed on 0.037 mm copper grids and stained with a 2.0 g/100 mL uranyl acetate aqueous stain (EMS, Hatfield, PA) to provide contrast under magnification. Excess liquid on the grid was removed with filter paper and the grid was allowed to dry before viewing under 50,000 times magnification. Observations were performed at 100 kV.

2.5. Entrapment Efficiency (EE)

The amount of active compound (eugenol and *trans*-cinnamaldehyde) entrapped in the inclusion complex particles was determined spectrophotometrically at 280 nm. For each type of inclusion complex, 5 mg of sample was dissolved in 5 mL of 95 g/100 mL acetonitrile and left for 24 h after being well mixed to allow enough time for all entrapped active compound to be in solution. Before measurement, the solutions were centrifuged at 3200 × g for 15 min to remove any BCD from the solution, leaving only the active compound. The *EE* was calculated (Gomes et al., 2011):