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Research paper

Antioxidant and antimicrobial properties of encapsulated guava leaf oil in hydroxypropyl-beta-cyclodextrin

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ABSTRACT

The essential oil from guava leaves has low physicochemical stability and low solubility in water, what limits its application in food formulations. This study aimed to characterize the physicochemical properties and the antioxidant and antimicrobial activities of encapsulated guava leaf oil in hydroxypropyl-β-cyclodextrin (HPβCD). Inclusion complex formation of guava leaf oil and HPβCD was determined by several techniques. Antioxidant activity of encapsulated guava leaf oil was more stable to sunlight exposure than free guava leaf oil by 26–38%. The antibacterial activity of guava leaf oil against *Staphylococcus aureus* and *Escherichia coli* was improved by 4 and 2 times after encapsulation in HPβCD, respectively.

1. Introduction

Guava (*Psidium guajava* L.) has long been used as a traditional medicine because of its biological properties (Jaiarj et al., 1999; Lozoya et al., 2002; Oh et al., 2005). The essential oil from guava leaves is responsible for antiproliferation, antioxidant and antimicrobial activities (Sacchetti et al., 2005; Manosroi et al., 2006). Limonene, β-caryophyllene, 1,8-cineole and α-pinene are the major constituents (Hsin-Chun et al., 2007; Ogunwande et al., 2003). However, its low solubility in water limits contact with food pathogens in aqueous matrices (Kalemba and Kunicka, 2003). Besides, some active compounds in essential oils are sensitive to chemical modifications under the effect of external factors such as temperature, light or oxygen (Dima et al., 2014).

The use of cyclodextrins for encapsulation can protect the essential oils (Hedges et al., 1995; Qi and Hedges, 1997) and improve their aqueous solubility in food (Helena and Cabral, 2010). Cyclodextrins are cyclic oligosaccharides of glucopyranosyl units linked by α-(1,4) bonds (Schmann and Schollmeyer, 2002). The widely used natural cyclodextrins are α-, β- and γ-cyclodextrin consisting of 6, 7 and 8 glucopyranose units, respectively. Cyclodextrins have a unique structure with a hydrophobic cavity and a hydrophilic surface, which can form inclusion complexes with a wide variety of guests. Cyclodextrins can be used to enhance the solubility of insoluble compounds, stabilize labile guests against oxidation, control volatility and sublimation, modify taste by masking off flavours, entrap odours and control the releasing of drugs and flavours (Marques, 2010). Among cyclodextrins, β-cyclodextrin is the most applicable, because of its suitable cavity size for common guests with molecular weights between 200 and 800 Da, availability, and reasonable price (Waleczek et al., 2003). Although β-cyclodextrin can be used with many guests, its solubility in water is low (1.8 g in 100 mL water at 25 °C). Therefore, cyclodextrin derivatives have been developed. Hydroxypropyl-β-cyclodextrin (HPβCD) is relatively high aqueous soluble (above 60 g in 100 mL water at 25 °C), with low toxicity, and satisfactory inclusion ability (Garnero et al., 2010). The aim of this study was to characterize the physicochemical, antioxidant and antibacterial properties of guava leaf essential oil encapsulated in HPβCD.

2. Materials

2.1. Microorganisms

Staphylococcus aureus and *Escherichia coli* (Microorganisms Collection of Department of Industrial Biotechnology, Faculty of Agro-Industry, Prince of Songkla University, Thailand) were respectively used as gram-positive and negative bacteria for the antimicrobial activity tests. They were cultivated in Tryptic Soy Broth (TSB) at 37 °C

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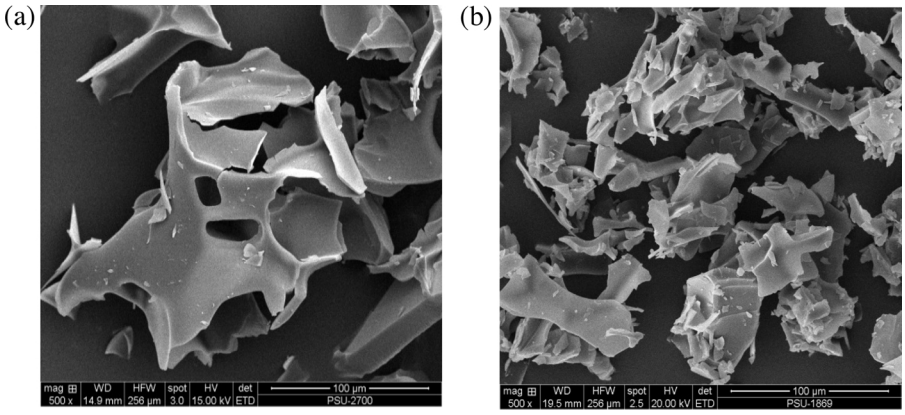

Fig. 1. SEM micrographs of free HPβCD (a) and encapsulated guava leaf oil (b) at 500 times magnification. The samples used were in powder form derived from inclusion complex formation via freeze-drying technique.

with shaking at 200 rpm for 24 h. All bacteria were maintained at −20 °C in 25% (v/v) glycerol.

2.2. Chemicals

Hydroxypropyl-β-cyclodextrin (HPβCD), 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and butylated hydroxytoluene (BHT) were purchased from Sigma-Aldrich (Steinheim, Germany). Guava leaf essential oil was purchased from Botanicessence (Bangkok, Thailand). Limonene was purchased from Sigma-Aldrich (Steinheim, Germany).

3. Methods

3.1. Composition in essential oils of guava leaf oil

Essential oils in guava leaf oil prepared from *P. guajava* L. were identified and quantified by following the GC–MS method used by Soliman et al. (2016).

3.2. Inclusion complex formation of HPβCD and guava leaf oil

The inclusion complex of HPβCD and guava leaf oil was prepared via the freeze-drying method (Karathanos et al., 2007). Therefore, 0.5 g of oil was slowly added to an aqueous solution of 5 g of HPβCD in 25 mL of water. The mixture was left for 24 h in a sealed container under stirring at room temperature and protected from the light, filtered through 0.45 μm PTFE filters (IC Millex-LH, Millipore, Billerica, MA), frozen at −20 °C, 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 powder was washed with acetonitrile and dried in a low temperature incubator at 25 °C. The products were stored in a sealed container inside a desiccator until use. The encapsulation efficiency was calculated compared to the standard curve of free guava leaf oil according to the method described by Bae and Lee (2008).

3.3. Characterization of encapsulated guava leaf oil

All the following methods were selected with the purpose of understanding changes in particle morphology by SEM, in molecular structure by FT-IR and UV–vis spectrophotometry, and in the stability by phase solubility studies of the guava leaf oil with encapsulation in HPβCD.

3.3.1. Morphological examination

The particle morphology of the encapsulated guava leaf oil was examined using a Quanta 250 Scanning Electron Microscope (SEM) (Quanta 250, Netherland). The samples (free HPβCD and encapsulated guava leaf oil powders) were fixed on aluminum stubs with double adhesive tape and vacuum coated with a fine layer of gold before viewing under 500 times magnification. Observations were carried out with voltage acceleration of 20 kV (Guimaraes et al., 2015).

3.3.2. FT-IR analysis

The FT-IR spectra of HPβCD, free guava leaf oil and encapsulated guava leaf oil were collected from 400 to 4000 cm^{-1} using a Nicolet 550-II FT-IR spectrophotometer (Nicolet, USA) with 32 scans at a resolution of 4 cm^{-1}. The HPβCD and encapsulated guava leaf oil powders were diluted with potassium bromide (KBr) powder at a mass ratio of 1:100, grounded and pressed to discs of 8 mm diameter. A drop of guava leaf oil sample was spread uniformly on a piece of KBr window and nipped with another piece of KBr window. FT-IR spectra were analyzed by the spectrophotometer software (OMNIC 5.2) (Gomes et al., 2014; Wang et al., 2011).

3.3.3. UV–vis spectroscopy analysis

The formation of inclusion complexes of guava leaf oil and HPβCD was studied by UV–vis spectrophotometry (Biochrom, LIBRA-S22, UK). Guava leaf oil was dissolved in acetonitrile (0.5 mg/mL). HPβCD, encapsulated guava leaf oil, and a 4:1 mixture of HPβCD and the essential oil were mixed with acetonitrile at 5 mg/mL and shaken for 10 min. The supernatant was separated by centrifugation, diluted by 100 times in acetonitrile, and scanned in the range of 200–400 nm to obtain the UV–vis absorption spectrum (Liu et al., 2013; Wang et al., 2011).

3.3.4. Phase solubility study

Phase solubility plots were used to classify and evaluate the stability of the inclusion complexes (Higuchi and Connors, 1965). An excess of guava leaf oil was added to 10 mL aqueous solutions of HPβCD ranging in concentrations from 0 to 10 mmol/L, incubated at 25 and 35 °C for 24 h, with shaking at 200 rpm, and filtered through 0.45-μm PTFE filters (IC Millex-LH, Millipore, Billerica, MA). The quantity of guava leaf oil remaining in solution was measured spectrophotometrically at 214.5 nm and compared to a standard curve of limonene. The quantity of guava leaf oil in the solution was plotted against HPβCD concentration. The stability constant, K_s (L/mol), was calculated from the slope and intercept of the plot according to the equation:

$$K_s = \frac{\text{slope}}{\text{intercept} \bullet (1 - \text{slope})} \quad (1)$$

where K_s (L/mol) is a stability constant, intercept (mmol/L) is the dissolved guest in the aqueous complexation medium when no cyclodextrin is present.

3.4. Evaluation of the antioxidant activity of encapsulated guava leaf oil

The samples (free and encapsulated guava leaf oil) were exposed to sunlight for 12 h. The antioxidant activity was determined by 1,1-