Food Hydrocolloids xxx (2016) 1–8

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Physico-chemical characterization and evaluation of bio-efficacies of black pepper essential oil encapsulated in hydroxypropyl-beta-cyclodextrin

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ARTICLE INFO

Article history:
Received 24 October 2016
Received in revised form
11 November 2016
Accepted 11 November 2016
Available online xxx

Keywords:
Black pepper oil
Hydroxypropyl-beta-cyclodextrin
Encapsulation characterization
Encapsulated oil bio-efficacy

ABSTRACT

Encapsulation of essential oils with cyclodextrins can protect their active compounds from environmental conditions and improve their aqueous solubility, hence increasing their functional capabilities as additives. The purpose of this study was to characterize the physico-chemical properties and bio-efficacies, antioxidant and antibacterial activities, of the encapsulated black pepper essential oil in hydroxypropyl-β-cyclodextrin (HPβCD), in comparison with the major ingredient in the oil, β-caryophyllene. The difference in encapsulation efficiency of the pure compound and the black pepper oil results from the presence of other components in the black pepper oil such as limonene, δ-3-carene and pinene. Although the inclusion complexes increase their stability, they gave slightly lower antioxidant activity as a result of the HPβCD was blocking the functional groups of active compounds during reaction with DPPH radicals. Instead, after encapsulated in HPβCD, the antibacterial activity of black pepper oil was improved by 4 times against both *S. aureus* and *E. coli*.

1. Introduction

Black pepper (*Piper nigrum* L.) is considered the king of spices because of its pungent of piperine (Srinivasan, 2007). It can be used for different purposes such as medicine, human dietaries, preservatives and biocontrol agents (Awen, Ganapati, & Chandu, 2010; Hussain, Naz, Nazir, & Shinwari, 2011; Srinivasan, 2007). It has been already reported that essential oil from black pepper possesses antioxidant (Singh, Marimuthu, Catalan, & de Lampasona, 2004) and antimicrobial activities (Dorman & Deans, 2000). Black pepper oil is basically composed of terpenes which have been found to be β-caryophyllene, limonene, δ-3-carene and pinene (Menon, Padmakumari, & Jayalekshmy, 2003; Singh et al., 2004). The major component of black pepper oil was found to be β-caryophyllene (Menon et al., 2003; Singh et al., 2004). Nevertheless, some active compounds in essential oils are sensitive towards the chemical modification under effect of some external factors such as: temperature, light, oxygen etc. (Dima et al., 2014). Besides, to apply with food products, an extremely low flavor threshold of essential oils can drastically change the sensory properties of foods, and highly water insoluble may have limited contact with pathogens (Kalemba & Kunicka, 2003).

The use of cyclodextrins for the essential oils encapsulation can protect the active compounds of essential oils from environmental conditions (Hedges, Shieh, & Sikorski, 1995, pp. 60–73; Qi & Hedges, 1997, pp. 231–243) and improve the aqueous solubility of essential oils for increasing their capacity to functionalize the products in which it is used as additive (Helena & Cabral, 2010). Cyclodextrin (CD) are cyclic oligosaccharides consisting of glucopyranosyl units linked by α-(1,4) bonds (Schmann & Schollmeyer, 2002). The widely used natural cyclodextrins are α-, β- and γ-cyclodextrin consisting of 6, 7 and 8 glucopyranose units, respectively. The cyclodextrin molecules have a unique structure with a hydrophobic cavity and a hydrophilic surface which can form inclusion complex with a wide variety of guests. Among those cyclodextrins, β-cyclodextrin is the most widely applicable kind because of its suitable cavity size for common guests with

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http://dx.doi.org/10.1016/j.foodhyd.2016.11.014
0268-005X/

molecular weights between 200 and 800 g/mol and its availability and reasonable price (Waleczek, Marques, Hempel, & Schmidt, 2003). In some cases, there is a need to enhance water solubility of β-cyclodextrin by adding the hydroxyalkyl groups on the β-cyclodextrin surface. A hydroxyalkylated or hydroxypropyl-β-cyclodextrin derivative (HPβCD) is relatively high aqueous solubility with low toxicity and satisfactory inclusion ability (Garnero, Zoppi, Genovese, & Longhi, 2010).

The purpose of this study was to characterize the physico-chemical properties and bio-efficacies, antioxidant and antibacterial activities, of the encapsulated black pepper essential oil in hydroxypropyl-β-cyclodextrin (HPβCD).

2. Materials

2.1. Microorganisms

The indicator bacteria used for testing antimicrobial activity were *Staphylococcus aureus* (representative for gram-positive bacteria) and *Escherichia coli* (representative for gram-negative bacteria). These strains were provided from the Microorganisms Collection of Department of Industrial Biotechnology, Faculty of Agro-Industry, Prince of Songkla University, Thailand. The indicator bacteria were cultivated in Tryptic Soy Broth (TSB) and incubated at 37 °C 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). Black pepper oil was purchased from Botanicessence (Bangkok, Thailand). β-caryophyllene was purchased from Sigma-Aldrich (Steinheim, Germany).

3. Methods

3.1. Encapsulation of black pepper essential oil

Black pepper oil was encapsulated in HPβCD through inclusion complex formation. The inclusion complex was prepared via the freeze-drying method (Karathanos, Mourtzinos, Yannakopoulou, & Andrikopoulos, 2007). The 0.5 g of black pepper oil was slowly added to an aqueous solution of hydroxypropyl-β-cyclodextrin (HPβCD) (5 g of HPβCD in 25 mL of water). The mixture was left in a sealed container under stirring at room temperature (~25 °C) and protected from the light for 24 h. The encapsulated particles dissolved in the solution were separated from the unencapsulated particles by filtration through 0.45-mm PTFE filters (IC Millex-LH, Millipore, Billerica, MA). The resulting solution containing encapsulated particles was 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 (to remove particles attaching on the HPBCD surface) and dried in a low temperature incubator at 25 °C. The product was stored in sealed container inside a desiccator until use. The encapsulation efficiency was calculated compared to the standard curve of black pepper oil, taking into consideration the non-encapsulated oil, according to the method described by Bae and Lee (2008).

3.2. Characterization of encapsulated black pepper 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 black pepper oil with encapsulation in HPβCD.

3.2.1. Morphological examination

The particle morphology of the encapsulated black pepper oil was examined using a Quanta 250 Scanning Electron Microscope (SEM) (Quanta 250, Netherland). The samples (powder of encapsulated black pepper oil and free HPβCD) 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 15 Kv (Guimaraes et al., 2015).

3.2.2. FT-IR analysis

The FT-IR spectra of free HPβCD, free black pepper oil and encapsulated black pepper 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 samples (powder of free HPβCD and encapsulated black pepper oil) were diluted with potassium bromide (KBr) powder at a mass ratio of 1:100. Then they were ground and pressed to discs of diameters of 8 mm. A drop of black pepper oil sample was spread on a piece of KBr window uniformly then nipped with another piece of KBr window. FT-IR spectra were analyzed by the spectrophotometer software (OMNIC 5.2) (Gomes, Petito, Costa, Falcao, & Araujo, 2014; Wang, Li, Si, & Chen, 2011).

3.2.3. UV–vis spectroscopy analysis

The formation of black pepper oil-HPβCD inclusion complex was demonstrated by a UV–vis spectrophotometer (Biochrom, LIBRA-S22, England). Black pepper oil (0.5 mg/mL) was dissolved in acetonitrile. The HPβCD, physical mixture (HPβCD:black pepper oil = 4:1 in a mass ratio) and encapsulated black pepper oil (inclusion complex) were prepared in acetonitrile (5 mg/mL), and then the mixture was shaken for 10 min. The supernatant was separated by centrifugation and then 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).

3.2.4. Phase solubility study

Classification of inclusion complex formation and stability of the inclusion compounds were evaluated by phase solubility study (Higuchi & Connors, 1965). An excess of black pepper oil was added to 10 mL aqueous solutions of HPβCD ranging in concentration from 0 to 10 mmol/L and incubated at 25 and 35 °C for 24 h with shaking at 200 rpm. The solution was filtered through 0.45-mm PTFE filters (IC Millex-LH, Millipore, Billerica, MA) prior to measurement to remove any insoluble material. The quantity of black pepper oil remaining in solution was measured spectrophotometrically at 205 nm and compared to a standard curve of a major component of black pepper oil, β-caryophyllene. The quantity of black pepper oil in the solution was plotted against HPβCD concentration.

The inclusion complex formation was classified according to the pattern of the plot between concentration of HPβCD and solubilized guests with the presence of HPβCD in solution. The stability constant, K_s (L/mol), was calculated from slope and intercept of the plot following Eq. (1):

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

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.

Please cite this article in press as: Rakmai, J., et al., Physico-chemical characterization and evaluation of bio-efficacies of black pepper essential oil encapsulated in hydroxypropyl-beta-cyclodextrin, *Food Hydrocolloids* (2016), http://dx.doi.org/10.1016/j.foodhyd.2016.11.014