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Antimicrobial and antioxidant activities of carvacrol microencapsulated in hydroxypropyl-beta-cyclodextrin

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ABSTRACT

Carvacrol is a hydrophobic compound that exhibits high antimicrobial and antioxidant activities. Cyclodextrins are used to increase solubility and dissolution through water-insoluble compounds inclusion into their hydrophobic cavities. Hence, this study aimed to characterize carvacrol inclusion complexes with hydroxypropyl-beta-cyclodextrin (HPBCD) by establishing the physico-chemical properties and evaluate their antimicrobial and antioxidant properties. Inclusion complexes were prepared by kneading (KN) and freeze-drying (FD) methods. Entrapment efficiency was 78.09 ± 1.24% for KN, and 83.74 ± 1.15% for FD. Polydispersity index was greater than 0.2 for both methods. Particle size for KN and FD were 0.360 ± 0.003 and 0.377 ± 0.007 μm, respectively. Carvacrol–HPBCD antimicrobial activity was higher ($P < 0.05$) than for free carvacrol for both bacteria, *Escherichia coli* K12 and *Salmonella enterica* serovar Typhimurium LT2, indicating that HPBCD increased water solubility and consequently increased contact between carvacrol and bacteria in medium. Antioxidant activity was lower ($P < 0.05$) for inclusion complexes indicating HPBCD makes carvacrol less available to react with free radical. The stability study indicated that light did not affect ($P > 0.05$) degradation, indicating that the microparticles were stable throughout storage. Therefore, carvacrol–HPBCD complexes may have important applications in the food industry as stable antimicrobial systems.

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1. Introduction

Recent outbreaks of foodborne pathogens such as *Escherichia coli* O157:H7 and *Salmonella* spp. continue to drive a search for innovative ways to inhibit microbial growth in foods while maintaining quality, freshness, and safety (Jin & Zhang, 2008). Recently, there has been a growing interest in natural antimicrobial products due to their availability, fewer side effects, or toxicity as well as better biodegradability as compared to the available preservatives (Kalemba & Kunicka, 2003). One such possibility is the use of essential oils (EOs) as antibacterial additives (Burt, 2004).

Carvacrol is a phenolic compound isomeric with thymol and the major constituent (50–86%) of the essential oil fraction of oregano and thyme plants, and can be obtained from the genera *Origanum*, *Thymus*, *Coridothymus*, *Thymbra*, *Satureja* and *Lippia* (Baser, 2008; Kulisic, Radonic, Katalinic, & Milos, 2004). It exhibits high antimicrobial and antioxidant activities and is generally recognized as a safe food additive (Kalemba & Kunicka, 2003; Undeger, Basaran, Degen, & Basaran, 2009). It is frequently used in several products as a flavoring and/or as an antimicrobial agent, showing a broad spectrum of activities against bacteria, yeasts and fungi (Knowles, Roller, Murray, & Naidu, 2005). Such microorganisms include *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella* Typhimurium (bacteria); *Candida albicans*, *Saccharomyces cerevisia*, *Candida tropicalis* (yeast); *Aspergillus niger*, *Botrytis cinerea*, and *Fusarium moniliforme* (fungi) for example (Baser, 2008). However, carvacrol is highly volatile and chemically labile component as a result of oxidation, chemical interactions, or volatilization (Seo, Min, & Choi, 2010). Besides, due to its poor water solubility and the requirement of high concentrations to reach a therapeutic effect, the efficiency of this compound in treatment is limited (Mastelic et al., 2008).

Encapsulation techniques have been extensively used during the last decades in the food industry since the encapsulated materials can be protected from moisture, heat or extreme conditions, thus enhancing their stability and maintaining viability (Gibbs, Kermasha, Alli, & Mulligan, 1999). A relevant method that

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has been developed for encapsulation is by using cyclodextrins (CD) (Cevallos, Buera, & Elizalde, 2010). Cyclodextrins are cyclic oligosaccharides consisting of six (α-cyclodextrin), seven (β-cyclodextrin), eight (γ-cyclodextrin) or more glucopyranose units linked by α-(1,4) bonds (Del Valle, 2004). They are enzymatically modified starch molecules shaped like a hollow truncated cone (Hedges, Shieh, & Sikorski, 1995). CDs are used to improve bioavailability by increasing solubility and/or dissolution through inclusion of water-insoluble compounds into their hydrophobic cavities, improving the stability, increasing the permeability of water-insoluble compounds, and reducing drug toxicity by making the compound effective at lower doses. Moreover, they have stabilization of labile guests against the degradative effects of oxidation, visible or UV light, and heat (Del Valle, 2004).

Hydroxypropyl-β-cyclodextrin (HPBCD) is a hydroxyalkylated β-CD derivative that combines relatively high water solubility with low toxicity and satisfactory inclusion ability (Garnero, Zoppi, Genovese, & Longhi, 2010). As the first approved CD derivatives by Food and Drug Administration (FDA), HPBCD has applications in food and agriculture (Szente & Szejtli, 2004; Yuan, Jin, & Li, 2008). Furthermore, it has been widely studied as a carrier for delivery of drugs (Liu, Lo, Tsai, & Cham, 2010; Tsao et al., 2012). However, there are few studies about encapsulation of essential oils using HPBCD and their physico-chemical properties.

Marques (2010) reviewed several techniques that are used to form cyclodextrin complexes, e.g., kneading, co-precipitation, heating in a sealed container, freeze drying, spray drying and supercritical fluid technology. Among them, kneading method, also known as slurry complexation, is a method that requires small amount of solvent in the preparation and gives a very good yield of inclusion. Consequently, it is conducive to a more easily scaled-up process and lower production costs (Hedges et al., 1995). Freeze drying is another method that produces a powdered sample in a very good yield of inclusion formation. The low temperature minimizes the loss of extremely volatile guests. This method is especially useful for heat labile guests and soluble complexes such as hydroxypropylated cyclodextrin complexes (Del Valle, 2004).

Thus, the objective of this study was to prepare and characterize the inclusion complexes of carvacrol with hydroxypropyl-β-cyclodextrin (HPBCD) using kneading and freeze-drying method to evaluate their antimicrobial effectiveness and antioxidant properties. A stability study was also carried out to evaluate the influence of light over the antioxidant activity.

2. Materials and methods

2.1. Materials

Carvacrol 98% was purchased from Sigma Aldrich Co. (St. Louis, MO, USA) and hydroxypropyl-β-cyclodextrin (HPBCD, average molar substitution = 4.2; average FW = 1379) was purchased from Acros Organics (Geel, Belgium). Tryptic soy agar (TSA), tryptic soy broth (TSB) and peptone for bacterial growth and enumeration were purchased from Becton, Dickinson and Co. (Franklin Lakes, NJ, USA). Tween 20 was obtained from VWR (West Chester, PA). HPLC-grade acetonitrile was purchased from EMD Chemicals (Darmstadt, Germany). For the antioxidant activity determination, potassium peroxydisulfate 99% was purchased from Alfa Aesar (Ward Hill, MA, USA). ABTS, 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) and diammonium salt were obtained from AMRESCO (Solon, OH). Trolox (6-hydroxy-2, 5, 7, 8-tetramethyl-chroman-2-carboxylic acid) from Tokyo Chemical (Tokyo, Japan) was used as an antioxidant standard. All other reagents were of analytical grade.

2.2. Preparation of hydroxypropyl-β-cyclodextrin inclusion complexes

Kneading and freeze-drying methods were used to obtain inclusion complexes. For kneading method (Manolikar & Sawant, 2003) the inclusion complexes of carvacrol–HPBCD were prepared using a 1:1 molar ratio. Approximately 0.90 g of carvacrol and 8.25 g of HPBCD were transferred to a mortar with a small volume of absolute ethanol (3 mL) and kneaded for 50 min. The pasty mass obtained was kept in a desiccator under vacuum for 48 h at room temperature to dry and then weighed and stored in a desiccator at −20 °C until further use.

For freeze-drying method (Karathanos, Mourtzinos, Yannakopoulou, & Andrikopoulos, 2007), carvacrol was dispersed in 25 mL of HPBCD aqueous solution (239 mmol/L − 8.25 g) in a 1:1 molar ratio and magnetically stirred at room temperature (25 °C) protected from light for 48 h to allow complex formation and prevent loss of volatiles to the atmosphere. After mixing, the solution was frozen at −20 °C and then lyophilized at −50 °C and 1.09 Pa in a Labconco Freeze Dryer-5 (Kansas City, MO, USA) for approximately 48 h. Finally, the lyophilized samples were weighed and stored in a desiccator at-20 °C until further use.

2.3. Differential scanning calorimetry (DSC)

DSC studies were performed using a differential scanning calorimeter model Q20 (TA Instruments, New Castle, DE, USA) at the Polymer Science Center (Texas A&M University, College Station, TX, USA). Samples of free carvacrol, HPBCD and their inclusion complexes were accurately weighed (~2 mg) and placed in aluminum pans (40 μL) with one hole in their lids. The instrument was calibrated using zinc and indium metals before sample testing. The specimens were heated from room temperature to 120 °C under nitrogen atmosphere at 90 °C/min, remained at 120 °C for 1 min, and then heated up to 420 °C at 10 °C/min (Mourtzinos, Kalegeroupoulos, Papadakis, Konstantinou, & Karathanos, 2008).

2.4. Entrapment efficiency (EE) and drug loading (DL)

The amount of carvacrol entrapped in the inclusion complex particles was determined spectrophotometrically (model Genesys 10S UV–Vis, Thermo Scientific, Madison, WI, USA) at 275 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 48 h after being well mixed to allow enough time for all entrapped carvacrol to be in solution. A standard curve of carvacrol was prepared with concentrations ranging between 2.5 and 30 μg/mL under the same conditions. The EE and DL were calculated according to Equations (1) and (2), respectively (Gomes, Moreira, & Castell-Perez, 2011; Iannitelli et al., 2011):

$$EE = \frac{\text{amount of active compound entrapped}}{\text{initial active compound amount}} \times 100 \quad (1)$$

$$DL = \frac{\text{amount of active compound entrapped}}{\text{amount of particles produced}} \times 100 \quad (2)$$

2.5. Particle size analysis and morphology

The average particles size and polydispersity indexes (PDI) for each type of inclusion complex particle were measured using a Delsa™ Nano C Particle Size Analyzer (Beckman Coulter, Brea, CA, USA). The particles were suspended in distilled water at a 1:3 ratio (w/v) in 1 cm path length plastic cuvettes at scattering angle of 165°,