Materials Science & Engineering C 94 (2019) 139–149

Contents lists available at ScienceDirect

Materials Science & Engineering C

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

Microencapsulation of garlic oil by β-cyclodextrin as a thermal protection method for antibacterial action

Raquel Piletti[a], Micheli Zanetti[b], Guilherme Jung[b], Josiane Maria Muneron de Mello[c,d], Francieli Dalcanton[c], Cintia Soares[a], Humberto Gracher Riella[a], Márcio Antônio Fiori[c,d,*]

[a] *Post-Graduate Program in Chemical Engineering, Universidade Federal de Santa Catarina (UFSC), Florianópolis 88040-900, SC, Brazil*
[b] *Department of Food Engineering, Universidade Comunitária da Região de Chapecó (UNOCHAPECÓ), Chapecó 89809-000, SC, Brazil*
[c] *Post-Graduate Program in Technology and Management of the Innovation, Universidade Comunitária da Região de Chapecó (UNOCHAPECÓ), Chapecó 89809-000, SC, Brazil*
[d] *Post-Graduate Program in Environmental Science, Universidade Comunitária da Região de Chapecó (UNOCHAPECÓ), Chapecó 89809-000, SC, Brazil*

ARTICLE INFO

Keywords:
Antibacterial materials
Natural antibacterial materials
Microencapsulation of natural oil
Microencapsulation of garlic oil
β-Cyclodextrin

ABSTRACT

The present study investigated the encapsulation process of garlic oil in β-cyclodextrin (βCD) and the antibacterial properties of the βCD-garlic oil complex against *Escherichia coli* and *Staphylococcus aureus*. The encapsulation method increased the thermal stability of garlic oil with a formation constant (Kc) value of 253.78 $L \cdot mol^{-1}$ for of the βCD-garlic oil complex, which confirmed the success of the encapsulation process. Scanning electron microscopy analysis showed that the dimensions of the structures formed by the inclusion complex of βCD-garlic oil had values ranging from 5 to 10 μm. After thermal treatment of the βCD-garlic oil complex at 60 °C for 1 h, the complex retained significant antibacterial action. The minimum inhibitory concentration (MIC) and agar diffusion results showed that the microcapsules containing 81.73 $mmol \cdot L^{-1}$ garlic oil exhibited excellent antibacterial action.

1. Introduction

Natural extracts and essential oils (EOs) have been studied for many years because of their antimicrobial properties and can be widely used in the industrial sector, especially in the food area [1]. Because of their inherent nature, EOs are frequently used in food products as an antimicrobial additive because they meet the requirements of consumers that prefer foods without synthetic preservatives [2].

Studies have shown that garlic oil is one of the most commonly used EOs for the control of growth of microorganisms [3]. Indu et al. reported that garlic oil has important antimicrobial properties in addition to allicin, and other authors have shown that garlic oil has excellent antibacterial activities with different concentrations and in different bacterial strains, such as *Escherichia coli* [4]. Fratianni et al. reported that garlic oil has excellent antibacterial activity with *Listeria monocytogenes*, *Salmonella enteritidis*, *Escherichia coli* and *Staphylococcus aureus* [5].

Garlic oil has excellent antibacterial properties but is prone to degradation by oxygen, light, heat, moisture and other aggressive agents. Thus, improving garlic oil stability will extend the antimicrobial action in applications. Antimicrobial properties of garlic oil are not efficient if the food is subjected to thermal treatments, exposed to oxygen atmosphere or applied as an antimicrobial additive in food packaging [6,7].

In this context, it is extremely challenging for food or packaging industries to use these natural oils as stable antimicrobial additives or as antioxidant additives. EOs can also easily change their sensory properties and are insoluble in water due to their lipophilic nature. These characteristics limit the interactions of EOs with photogenic agents and decrease the antimicrobial efficiency [2,7]. Due to these limitations, the majority of active packaging is manufactured with antimicrobial metallic nanoparticles or antimicrobial inorganic additives [8–14].

Garlic oil may be applied in many other areas if its thermal stability was improved. Garlic oil can be used as an additive in the textile industry to manufacture antibacterial tissues for use in hospitals and laboratories, and it can be used by the cellulose industry for the production of antibacterial papers. However, the application of the garlic oil in these processes is difficult because the natural oil is sensitive to thermal effects. Therefore, encapsulation of garlic oil molecules by nontoxic and thermal-resistant compounds may allow the application of

* Corresponding author at: Post-Graduate Program in Technology and Management of the Innovation and Post-Graduate Program in Environmental Sciences, Universidade Comunitária da Região de Chapecó (UNOCHAPECÓ), Chapecó 89809-000, SC, Brazil.
E-mail address: marciofiori@gmail.com (M.A. Fiori).

https://doi.org/10.1016/j.msec.2018.09.037
Received 17 October 2017; Received in revised form 30 August 2018; Accepted 11 September 2018
Available online 12 September 2018
0928-4931/

this oil in industrial processes involving relatively high temperatures. Cyclodextrins are compounds that have been used for this purpose to encapsulate many organic compounds.

Cyclodextrins are sugar structures that encapsulate hydrophobic components and can protect encapsulated components from oxidation and thermal degradation reactions. Therefore, encapsulation of garlic oil may reduce the volatility rate, increase thermal resistance, increase durability of antimicrobial properties and increase protection from oxidative reactions of garlic oil [2,15,16]. Cyclodextrins are a group of cyclic natural molecules of glycopyranose units linked by α(1–4) bonds. These oligosaccharides are obtained from the enzymatic degradation of starch under the action of the glycosyltransferase enzyme [17]. These starch derivatives are non-toxic ingredients that are not absorbed by the gastrointestinal tract and are completely metabolized by colonic microflora. In addition, α-, β- and γ-cyclodextrins are considered safe for use as food additives by the FDA [18–20].

Due to the hydrophobic cavity in the cyclodextrin molecules, in which substances can be "entrapped" to form inclusion complexes, cyclodextrins may host different compounds, generally with a stoichiometry of 1:1 [14]. The chemistry of the molecular inclusion represents a system of structural complexity where cyclodextrin is the host and the substrate are the guest, and they are held together by Van der Waals interactions, hydrophobic interactions and hydrogen bonds [21]. These characteristics make β-cyclodextrin molecules a good option for the encapsulation of many organic compounds and an excellent method for protecting EOs from degradation. This protection occurs due to the incorporation of essential oil molecules into the cone formed by the β-cyclodextrin molecules [2,22]. During microencapsulation, oil molecules (guest) interact with βCD molecules (host), and inclusion complexes are formed by the partial and complete hydrophobic and hydrophilic interactions between the molecules [2,20].

In this study, garlic oil was encapsulated in β-cyclodextrin molecules, and the thermal stabilities of the obtained garlic oil-β-cyclodextrin complexes were determined using various thermal analysis techniques. The antibacterial actions of the complexes were also investigated and correlated with thermal treatment conditions. Detailed thermal analysis revealed that the microencapsulation process protects the essential oil and that the antibacterial action is retained after several thermal treatments.

2. Experimental procedures

2.1. Preparation of the garlic oil-βCD complex

The preparation of garlic oil-βCD complexes was performed using a modified methodology proposed by Hill et al. [2]. To obtain garlic oil-βCD complexes, the following concentrations of garlic oil were added to distilled water containing a mass of 1.00 g of β-cyclodextrin (βCD) (Sigma Aldrich; W402826-100G; purity 98%): 245.20, 122.60, 81.73, 40.87, 27.24, 20.43, 16.32 and 13.62 mmol·L^{-1}. These solutions were prepared in a closed glass reactor to avoid loss of volatile compounds during agitation (Dist – Model THOLZ TDH) at temperature of 25 °C for 24 h. The solutions were then dried in a lyophilizer (LIOTOP Model L101) at −52 °C and a pressure of 62 μHg for 72 h until the water was completely removed.

After the lyophilization step, the garlic oil concentrations incorporated into the garlic oil-βCD complexes were quantified using UV–Vis spectroscopy (Scinco, Model SUV 2120) and analyzing the characteristic absorbance peak for garlic oil at a wavelength of 217 nm. The calibration curve was obtained from solutions of 90% v/v distilled water and 10% v/v dimethyl sulfoxide (DMSO) with garlic oil at concentrations ranging from 0.01 to 0.1 mmol·L^{-1}.

2.2. Determination of encapsulation constant

The encapsulation constant was determined from phase solubility studies according to the methods proposed by Higuchi and Connors [23] and Brandão et al. [24]. The βCD molecules were added at different concentrations (0 to 0.7 mmol·L^{-1}) in an aqueous solution with a constant concentration of the garlic oil and in excess (2 mmol·L^{-1}). After 24 h of agitation in a shaker at 25 °C, the solutions were filtered with a membrane and evaluated with UV–Vis. βCD molecules normally increase the solubility of garlic oil in aqueous solutions. Increasing βCD concentration in the aqueous solution (with garlic oil in excess) resulted in increasing amounts of solubilized garlic oil. In an aqueous solution containing excess garlic oil, the amount of solubilized garlic oil in the aqueous medium increases linearly with the increase of βCD concentration until a value limit. These procedures are used to study the phase solubility and to determine the encapsulation constant, which are important parameters for encapsulation processes [18–20].

The encapsulated garlic oil concentration was determined using UV–Vis spectroscopy for each solution at a wavelength of 217 nm. The intensity of this characteristic peak was used to determine the garlic oil concentrations in the complexes synthesized according to the calibration curve obtained in the previous step. Therefore, the solubility profiles were obtained from a plot of the garlic oil concentration versus βCD concentration.

The apparent stability constants (Kc) of the βCD-garlic oil complexes were determined from the slope and linear coefficients of the linear function used to fit the experimental data in the solubility profiles according to Eq. (1) as follows:

$$Kc = \frac{k}{So}(1 - k) \tag{1}$$

where S_0 (mol·L^{-1}) is the concentration of garlic oil solubilized in deionized water in the absence of βCD; and K is the slope of the linear function used to fit the experimental data in the solubility profiles [22].

2.3. Thermal treatment of garlic oil-βCD complexes

The garlic oil-βCD complexes were thermally treated at a temperature that was twice as high as the volatilization temperature of garlic oil as determined by TGA analysis. After lyophilization, the garlic oil-βCD complexes were dried in an oven (Quimis) at 80 °C for 2 h, and the garlic oil concentrations incorporated into the compounds synthesized were determined by UV–Vis spectroscopy (Scinco, Model SUV 2120) adopting the characteristic wavelength of 217 nm. The calibration curve was used to determine the garlic oil concentrations.

2.4. Thermogravimetric analysis (TGA)

To analyze the thermal stability of the garlic oil-βCD complexes and their components, thermogravimetric analysis (TGA) was used. These analyses were performed using a Schimadzu thermogravimetric analyzer (Model TGA 50) with a temperature range of 25 to 810 °C, a heating rate of 10 °C min^{-1} and under a nitrogen atmosphere with a flow rate of 50 mL·min^{-1}.

2.5. Fourier transform infrared (FTIR) spectroscopy

The synthesized compounds were analyzed by FTIR with a Schimadzu spectrometer (Model IR PRESTIGE-21) in the absorbance mode and wavelengths ranging from 400 to 4000 cm^{-1}. FTIR spectroscopy was applied to analyze the pure garlic oil sample, the pure β-cyclodextrin sample and the garlic oil-βCD complexes. Samples were prepared by mixing 2 mg of garlic oil, β-cyclodextrin or garlic oil-βCD complexes with 200 mg of KBr. The mixtures were pressed to form tablets with a thickness of 1 mm.

2.6. Scanning electron microscopy (SEM)

The morphology of β-cyclodextrin and garlic oil-βCD complexes was