Industrial Crops & Products 112 (2018) 281–289

Contents lists available at ScienceDirect

Industrial Crops & Products

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

Cinnamon oil-loaded composite emulsion hydrogels with antibacterial activity prepared using concentrated emulsion templates

Jingguang Wang[a,1], Yaozong Li[a,1], Yongfeng Gao[a], Zoufeng Xie[a], Muhua Zhou[a], Yinyan He[a], Hong Wu[b], Wuyi Zhou[a,c], Xianming Dong[a], Zhuohong Yang[a,*], Yang Hu[a,*]

[a] College of Materials and Energy, South China Agricultural University, Guangzhou 510642, China
[b] College of Life Sciences, South China Agricultural University, Guangzhou 510642, China
[c] Key Laboratory of Application Technology of Environmental Photocatalysis of Hunan Province, Changsha University, Changsha 410022, China

ARTICLE INFO

Keywords:
Cinnamon oil
Composite hydrogels
Concentrated emulsion
Sustained release
Antibacterial activity

ABSTRACT

Cinnamon oil (CMO) has a wide variety of promising applications due to its multifunctional activity, especially excellent antibacterial activity. However, CMO is highly unstable and volatile, which clearly limit its application fields. In this work, CMO is encapsulated in composite emulsion hydrogels to solve the above issue. The CMO-loaded composite emulsion hydrogels are facilely and effectively fabricated by free radical polymerization of acrylamide in the continuous phase of oil-in-water concentrated emulsion templates. Gelatin serves as the effective polymer emulsifier to prepare the CMO-in-water concentrated emulsions. The study results display that the CMO can be effectively loaded into the hydrogel matrix. And the microstructure and mechanical properties of the composite hydrogels can be easily tuned by changing internal phase fraction of the concentrated emulsion templates. Increasing internal phase fraction leads to the increase of pore sizes and the decrease of the Young's modulus and compressive stress. Moreover, the release studies indicate that the CMO-loaded composite hydrogels display the sustained CMO release profiles. Furthermore, the antibacterial assays verify that the CMO-loaded composite hydrogels demonstrate an excellent and long-term antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. All the results indicate that the CMO-loaded composite emulsion hydrogels prepared in this study can be applied as promising long-term antibacterial materials. Free radical polymerization based on concentrated emulsion templates is a promising alternative approach to fabricate antibacterial essential oil-loaded composite emulsion hydrogels, which could be acted as antibacterial materials.

1. Introduction

Polymer hydrogels as "soft-materials", contain substantial quantities of water in their three-dimensional polymeric networks (Komaiko and McClements, 2015; Hu et al., 2016a) have acquired increasing attention in extensive applications, such as drug delivery (Ooi et al., 2016; Ramin et al., 2017; Li et al., 2015), tissue engineering (Dou and Feng, 2017; Tseng et al., 2017), actuation (Zhao et al., 2017), absorption (Song et al., 2017), separation (Gumuscu et al., 2016) and sensation (Jia et al., 2016). Especially, hydrogels utilized in the development of drug delivery systems can encapsulate, protect and release drugs, which assist in improving the stability, prolonging the shelf life, maintaining the functional property and controlling the release behavior of drugs. Several approaches including freeze-thawing (Huang et al., 2016; Morariu et al., 2016), gas foaming (Gupta and Shivakumar, 2010; Gunathilake et al., 2017), emulsion templates (Chen et al., 2017; Zou et al., 2014; Zhou et al., 2013) and particulate leaching (Badhe et al., 2017; Chiu et al., 2013) have been proposed to prepare the hydrogels with porous structure (named as porous hydrogels). Recently, the emulsion template (especially oil-in-water type concentrated emulsion template) has received increasing interest in the preparation of porous hydrogels (Liu et al., 2017; Yi et al., 2016), because of its simplicity and versatility. The concentrated emulsions are a kind of unique emulsions with high internal phase fractions, and also named as high internal phase emulsions when the internal phase fractions are more than 74% of the total emulsion volumes (Menner et al., 2006; Hu et al., 2015). The concentrated emulsions can be stabilized by surfactants, biomacromolecules or solid particles (Zhang et al., 2016; Zeng et al., 2017; Venkataramani and Aichele, 2015; Li et al., 2013). The porous hydrogels are usually synthesized by solidifying the continuous external phase of concentrated emulsions and then removing the dispersed internal phase (oil phase) (Tan et al., 2017). However, the

* Corresponding authors.
E-mail addresses: yangzhuohong@scau.edu.cn (Z. Yang), huyang0303@scau.edu.cn (Y. Hu).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.indcrop.2017.12.022
Received 25 June 2017; Received in revised form 3 December 2017; Accepted 9 December 2017

removal disposal of the oil phase is relatively difficult and time-consuming. Furthermore, it is also a great waste of the oil phase because of direct removal in the post-treatment process. To solve the above issues, the hydrogels containing internal oil phase (coded as emulsion hydrogels) can be directly used as drug carriers (Singh et al., 2014; Satapathy et al., 2015). Specifically, the oil phase in the emulsion hydrogels can be served as the drug solvent, even that the functional oil (for example plant essential oil) as the oil phase can be acted as the active drugs.

Cinnamon oil (CMO), a kind of plant essential oil extracted from cinnamon barks, branches and leaves, is the main medicinal and edible components of cinnamon. CMO as raw material has promising applications in a wide variety of fields including cosmetics, biomedicine, food, pesticide and perfume, because of its favorable antibacterial activity, insect-repellent property, antioxidant effect and spectacular aromatic flavor (Ribeiro-Santos et al., 2017; Xie et al., 2017; Kim et al., 2013; Qiu et al., 2017). The CMO antibacterial test results have showed that CMO not any can effectively inhibit the growth of bacteria, but also availably inhibit the growth of molds and yeasts (Matan et al., 2006; Kaskatepe et al., 2016; Xing et al., 2011), which indicates its broad-spectrum antibacterial activity and application as natural antibacterial agent. However, CMO is highly unstable in the undesirable environmental conditions in the presence of oxygen, light and heat, which clearly decrease its functional performance and greatly limit its application fields (Ayala-Zavala et al., 2008; Feng et al., 2017). To solve the above issues, several strategies have been developed to encapsulate and incorporate CMO in emulsions (Vandyousefi and Bhargava, 2017) and microcapsules (Felix et al., 2017). However, the emulsions are thermodynamic instability dispersed systems, which are easily agglomerate and demulsification, leading to the reduction of the amount and bioactivity of the encapsulated CMO. In addition, the CMO loading capacity in microcapsules is relatively low due to their micron sizes. Therefore, in this study CMO as the internal oil phase was encapsulated in emulsion hydrogels using the concentrated emulsion templates for obtaining stable CMO carriers with high loading capacity. The prepared emulsion hydrogels are supposed to decrease the volatility, improve the stability, lengthen the efficiency, and broaden the potential application fields of CMO. In addition, the functional CMO encapsulated in the emulsion hydrogels does not need to be removed out, which avoids the waste of oil phase.

In this study, we reported an effective approach to fabricate CMO-loaded composite emulsion hydrogels with antibacterial activity basing on the oil-in-water (O/W) concentrated emulsion templates. To be specific, we employed gelatin as polymer emulsifier to prepare O/W concentrated emulsions with acrylamide as the monomer in the water phase and the functional CMO as oil phase. Then, the CMO-loaded composite hydrogels were one-pot synthesized by free radical polymerization of acrylamide in the continuous phase of concentrated emulsion templates. The effects of internal phase fraction on the emulsion morphology, microstructure and mechanical properties of the composite hydrogels were evaluated in detail. In addition, the CMO release behavior and antimicrobial activity against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) of the composite hydrogels were also discussed. The O/W concentrated emulsion templates are supposed to an excellent route to prepare the CMO-loaded composite emulsion hydrogels with antibacterial activity, which are suitable to be used as antibacterial materials.

2. Experimental section

2.1. Materials

CMO was purchased from Jiangxi Jishui Lvxiang Spice Refinery, China. Gelatin was obtained from Shanghai Sinopharm Chemical Reagent Co., Ltd., China. Acrylamide and ammonium persulfate (APS) were supplied by Tianjin Fuchen Chemical Factory, China. *N*,*N*'-Methylene bisacrylamide (MBA) was provided with Tianjin Damao Chemical Reagent Co., Ltd., China. All chemicals were applied as received without any purification. The used water in this study was purified by filtration and deionization with a water purification apparatus.

2.2. Fabrication of composite emulsion hydrogels

The preparation procedures of CMO-loaded composite emulsion hydrogels were shown as follows. Firstly, 0.3 g of gelatin was added into 2.7 mL of distilled water and magnetic stirred at 45 °C to form the gelatin aqueous solution (10 wt%). Then 1 g of the monomer acrylamide and 20 mg of the cross-linker MBA (2 wt%, weight ratio of MBA/acrylamide) were dissolved into the above gelatin aqueous solution with the aid of ultrasound at ice-water temperature. After the mixture solution was excluded oxygen by inletting nitrogen stream for 30 s, 0.3 mL of the initiator APS solution (25 mg mL^{-1}) was added under shaking conditions to obtain the water phase. Afterwards, CMO (oil phase) was added to the water phase in batches and then shaken by a vortex machine at 3000 rpm for 20 s at every turn to obtain the concentrated emulsion with the internal phase volume fraction of 70%, 75%, 80% or 85%. Furthermore, the resulting emulsion was transferred into cylindrical glass tubes sealed with plastic wraps, and then polymerized in a 45 °C water bath for 24 h to form the CMO-loaded composite emulsion hydrogels. The general schematic illustration of the fabrication of CMO-loaded composite emulsion hydrogels is shown in Fig. 1.

2.3. Characterization

The concentrated emulsions were observed with an optical microscope (Carl Zeiss, German). The size distribution and mean diameters of the concentrated emulsions were measured with a Malvern Mastersizer 2000. The X-ray diffraction (XRD) test of the hydrogel samples was performed using an X'pert PRO diffractometer with a Cu Kα radiation (wavelength 0.154 nm). The Fourier transform infrared (FTIR) spectra of hydrogel samples were recorded via a German Vector-33 infrared instrument from 400 cm^{-1} to 4000 cm^{-1}. The thermal gravimetric analysis (TG) of the samples was carried out under N_2 via a thermoanalyzer from 40 to 750 °C with the heating rate of 10 °C min^{-1}. The differential scanning calorimetry (DSC) curves of the samples were obtained using a PerkinElme 8000 differential scanning calorimeter from 50 to 300 °C with the heating rate of 10 °C min^{-1} under N_2 protection. The microstructures of gel samples were observed using a Zeiss EVO 18 scanning electron microscope (SEM) at 10 kV. For the microstructure observation, the hydrogel samples were purified using ethanol to remove CMO and freeze-dried to obtain macroporous composite gels. After frozen with liquid nitrogen, the composite gels were sliced with a knife blade and coated with a thin gold layer to observe the microstructures using SEM. Several SEM micrographs of the macroporous gel samples were taken and the average pore size and pore size distribution were determined by measuring more than 80 pores from SEM images using the image analysis software (Nano Measurer 1.2 software). The porosity of the macroporous composite gels was measured at 25 °C according to the liquid displacement method described in our previous study (Hu et al., 2016b) with the use of hexane as displacement liquid. The compression properties of the as-prepared CMO-loaded hydrogel samples (15 mm in diameter and 10 mm in height) were determined with a Shimadzu universal material testing machine at room temperature. The compression tests were performed until break or the height reduction of 80% at the crosshead speed of 2 mm min^{-1}. The Young's modulus was obtained through calculating the slope of the initial linear portion of the compressive stress-strain curve. Each test was carried out at least in triplicate.