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Physical and antimicrobial properties of thyme oil emulsions stabilized by ovalbumin and gum arabic

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ABSTRACT

Natural biopolymer stabilized oil-in-water emulsions were formulated using ovalbumin (OVA), gum arabic (GA) solutions and their complexes. The influence of interfacial structure of emulsion (OVA-GA bilayer and OVA/GA complexes emulsions) on the physical properties and antimicrobial activity of thyme oil (TO) emulsion against *Escherichia coli* (*E. coli*) was evaluated. The results revealed that the two types of emulsions with different oil phase compositions remained stable during a long storage period. The oil phase composition had an appreciable influence on the mean particle diameter and retention of the TO emulsions. The stable emulsion showed a higher minimum inhibitory concentration (MIC), and the TO emulsions showed an improved long-term antimicrobial activity compared to the pure thyme oil, especially complexes emulsion at pH 4.0. These results provided useful information for developing protection and delivery systems for essential oil using biopolymer.

1. Introduction

Essential oils contain a complex mixture of nonvolatile and volatile compounds produced by aromatic plants as secondary metabolites which have antioxidant, antiradical, and antimicrobial properties (Bakkali, Averbeck, Averbeck, & Idaomar, 2008; Burt, 2004), and are classified by the United States Food and Drug Administration as generally recognized as safe (GRAS) (Weiss, Gaysinsky, Davidson, & McClements, 2009). They have been widely used as functional ingredients in food, cosmetic, and pharmaceutical applications (Bakkali et al., 2008; Cimanga et al., 2002). For example, TO has been shown to have inhibitory activities against various bacteria and yeasts (Gaysinsky, Davidson, McClements, & Weiss, 2008). However, direct incorporation of essential oils in food systems encounters many challenges due to their low water solubility and interactive binding with food components such as protein and lipids, which limits their utilization in aqueous-based foods and beverages.

A feasible way to improve essential oils dispersibility was to encapsulate essential oil within emulsion-based delivery systems. Good antimicrobial transport systems kept stably during 30 days storage periods and maintained good antimicrobial activity. Protein/polysaccharide emulsion possessed potential application value in food industries due to the good stability against flocculation and coalescence (Li, Fang, Phillips, & Al-Assaf, 2013; Niu et al., 2015). However, when the essential oil had good solubility in the aqueous phase it often induced Ostwald ripening (Kabalnov, 2001; Wooster, Golding, & Sanguansri, 2008). Previous research showed that a mixture of water insoluble oil and essential oil could inhibit Ostwald ripening. Water insoluble oils as inhibitors were usually a highly nonpolar and high molecular weight material, such as corn oil (Ziani, Chang, McLandsborough, & McClements, 2011), sunflower oil (Donsì, Annunziata, Vincensi, & Ferrari, 2012) and medium chain triglycerides (Liang et al., 2012). Weinbreck, Minor, and De Kruif (2004) studied the encapsulation and release property of lemon and orange essential oil by using the whey protein/gum Arabic complexes and found that large capsule (50–100 μm) showed greater release when compared with smaller under chewing. Liu et al. (2013) also investigated the release property of vitamin, which showed that the whey protein isolate/gum Arabic or carrageenan could better control the release of vitamin E and B_{12}, respectively. Nevertheless, the influence of interfacial structure of protein/polysaccharide emulsion on the antimicrobial activity of essential oil has not been established. This necessitated further work to explore the relationships between the interfacial structures, surface charge, stability of

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protein/polysaccharide emulsion and the release, antimicrobial properties of essential oil.

To study the possibility of blending OVA and GA to prepare stable thymol oil emulsion, we used TO as a model essential oil to form emulsions and used sunflower oil (SO) as ripening inhibitors. The influence of the interfacial structure of emulsion (bilayer and complexes emulsions) on the physical stability and antimicrobial activity of TO emulsions against *E. coli* was investigated. The results of this study could be useful for designing antimicrobial delivery systems with improved properties and consumer acceptance.

2. Materials and methods

2.1. Materials

Ovalbumin (lyophilized powder, molecular weight 42.7 kDa) was purchased from Amresco Chemical Co. (Boise, Idaho, USA). The composition of the powder was 90.3% total protein (% N × 6.25), 5.92% moisture, 0.2% fat and 2.82% ash.

Gum arabic was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The powder contained 9.2% moisture, 89.24% dry solid, and 4.91% (w/w) ash. Commercial sunflower oil (SO) was purchased from a local supermarket, and contained approximately 13.0, 26.0, and 61.0% (w/w) of saturated, monounsaturated, and polyunsaturated fats, respectively. All other chemicals were of analytical grade and were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Emulsion preparation

Mixtures of OVA and GA at ratio of 2:1 (0.6%, w/v) were obtained by dissolving each powder in deionized water containing 0.02% (w/v) sodium azide as an antimicrobial agent, stirring gently (500 rpm) at room temperature for 4 h, and then overnight at 4 °C to ensure biopolymer dissolution (Niu et al., 2014a, 2014b). Bilayer emulsions were prepared according to our recent report (Niu et al., 2015). Briefly, the OVA/GA mixtures were adjusted to pH 7.0 using 0.1 M NaOH, before homogenization. The oil-in-water (O/W) emulsions were prepared by dispersing 5% (v/v) SO in an OVA/GA mixture solution. Primary emulsions were prepared using an Ultra-Turrax blender (IKA T25 Basic, Staufen, Germany) at 11,000 rpm for 2 min. Fine emulsions were prepared by homogenization twice with a high-pressure homogenizer at 40 MPa through the homogenizer (APV1000, APV Co., Crawley, UK). The pH of the emulsion was adjusted with 0.1 M HCl or 0.1 M NaOH to the desired pH. Complexes emulsion: the OVA/GA mixtures solution were adjusted to different pH value, and then complexes emulsions were obtained by emulsification of oil with aqueous solutions of OVA/GA complexes according to above method.

2.3. Particle size measurements

Emulsions were prepared according to our earlier method (Niu et al., 2015). The droplet size distribution and mean particle diameter of the emulsions were determined using the laser diffraction apparatus Zetasizer nano ZS (Malvern Instruments Ltd., Malvern, UK). Emulsion samples were diluted 1:100 using deionized water of the appropriate pH. Samples were equilibrated for 60 s inside the instrument before data was collected at least 12 sequential readings. Reported z-averages were the result of three independent replicates.

2.4. ζ-Potential measurements

The ζ-potential of the emulsion samples were calculated by measuring the electrophoretic mobility of the droplets using a capillary electrophoresis cell (Zetasizer nano ZS, Malvern Instruments Ltd., Malvern, UK). The samples were diluted to a droplet concentration of approximately 1:100 using deionized water of the appropriate pH. Samples were equilibrated for 60 s inside the instrument before data were collected over at least 10 sequential readings and the Smoluchowsky mathematical model was used to convert the electrophoretic mobility measurements into the ζ-potential values (Qiu, Zhao, & McClements, 2014). All measurements were made in triplicate.

2.5. Encapsulation efficiency determination

The encapsulation efficiency (EE) of TO was determined according to the method described by Tao, Hill, Peng, and Gomes (2014) with slightly modification: 50 μL of emulsion were dissolved in 5 mL of 95 mL/100 mL acetonitrile in water and mixed for 48 h in order to ensure that all the TO active compound was released to solution. Before measurement, the solutions were centrifuged for 10 min at 6930*g* and were filtered through 0.22 μm Nylonfilters (VWR Intl., Radnor, PA., USA) to remove any insoluble substance from the solution. The optical density of solution was determined using an UV/vis spectrophotometer (WFJ 2000, UNICO, St. Louis, MO, USA) at 274 nm. Each experiment was performed in duplicate. The EE was calculated by Eq. (1):

$$\text{EE } (\%) = \frac{m_1}{m_2} \times 100 \quad (1)$$

where m_1 is the amount of TO encapsulation, and m_2 is initial amount of TO.

2.6. Retention determination

The fresh emulsions were placed in room temperature for 30 days and regularly take out a certain amount of the emulsion to determine the content of TO according to method described previously. The retention of TO was calculated by Eq. (2):

$$\text{Retention } (\%) = \frac{m_3}{m_1} \times 100 \quad (2)$$

where m_1 is the amount of TO encapsulation, and m_3 is the residue amount of the TO in the emulsion after storage.

2.7. Antimicrobial activity measurement

2.7.1. Determination of minimum inhibitory concentration

The MIC was determined according to the procedure of Hammer, Carson, and Riley (1999) with some modifications. Briefly, a series of 2-fold dilutions of bulk TO and TO emulsion were mixed with 15 mL Luria-Bertani culture (LB) in open glass beakers to make the final concentration gradient of TO of 12.5, 5, 2.5, 1.25, 0.625, 0.312, 0.156 μL/mL. Culture plates were solidified at room temperature for 30 min prior to inoculations with 5 μL spots containing approximately 10^4 CFU/mL of each organism onto the agar surface using an autopipet. LB culture with no oil and LB culture with pure oil were used as controls. Inoculated plates were incubated at 37 °C for 24 h. MICs against each strain were determined as the lowest concentration of TO and TO emulsion inhibiting the visible growth of test microorganism on the agar culture. Each experiment was repeated three times.