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Curcumin liposomes prepared with milk fat globule membrane phospholipids and soybean lecithin

Hong-Hao Jin,*[1] Qun Lu,*†[1] and Jian-Guo Jiang*[2]
*College of Food and Bioengineering, South China University of Technology, Guangzhou, 510640, China
†College of Food Science and Technology, Huazhong Agricultural University, Wuhan 470070, China

ABSTRACT

Using thin film ultrasonic dispersion method, the curcumin liposomes were prepared with milk fat globule membrane (MFGM) phospholipids and soybean lecithins, respectively, to compare the characteristics and stability of the 2 curcumin liposomes. The processing parameters of curcumin liposomes were investigated to evaluate their effects on the encapsulation efficiency. Curcumin liposomes were characterized in terms of size distribution, ζ-potential, and in vitro release behavior, and then their storage stability under various conditions was evaluated. The curcumin liposomes prepared with MFGM phospholipids had an encapsulation efficiency of about 74%, an average particle size of 212.3 nm, and a ζ-potential of −48.60 mV. The MFGM liposomes showed higher encapsulation efficiency, smaller particle size, higher absolute value of ζ-potential, and slower in vitro release than soybean liposomes. The retention rate of liposomal curcumin was significantly higher than that of free curcumin. The stability of the 2 liposomes under different pH was almost the same, but MFGM liposomes displayed a slightly higher stability than soybean liposomes under the conditions of Fe^{3+}, light, temperature, oxygen, and relative humidity. In conclusion, MFGM phospholipids have potential advantages in the manufacture of curcumin liposomes used in food systems.
Key words: curcumin, liposome, milk fat globule membrane, phospholipid, soybean lecithin

INTRODUCTION

Curcumin, one of the phenolic compounds derived from turmeric, is commonly used as a spice or food colorant. Curcumin has been shown a variety of biological activities, such as antitumor, antioxidant, anti-inflammatory functions, and the capacity of lowering blood pressure (Khar et al., 1999; Anand et al., 2007, 2008). However, curcumin is susceptible to basic pH, light, and temperature, which reduce its stability. The poor chemical stability and low bioavailability of curcumin limit its applications in food and pharmaceutical industries.

The preparation of curcumin liposomes can protect curcumin from unfavorable conditions. Liposomes, spherical bilayer vesicles, are amphipathic phospholipid bilayer membranes with an aqueous core inside and hydrophobic layer on the surface. Liposomes have been demonstrated to possess many typical advantages including biodegradability, biocompatibility, solubilization of poorly soluble compounds, and sustained-releasing capability (Kima and Baianua, 1991; Neethirajan and Jayas, 2011). In the food industry, liposome is often used to encapsulate nutraceuticals, food flavors, food colorants, and food antimicrobials (Keller, 2001; Taylor et al., 2005). The traditional liposomal materials are highly purified lecithin extracted from soybean or egg yolk. The lipid systems presented the benefit of low toxicity compared with polymeric materials.

Phospholipids are one of the main constituents of milk fat globule membranes (**MFGM**). The nutritional and functional importance of MFGM-derived phospholipids has attracted more and more researchers to apply it in their studies (Spitsberg, 2005; Dewettinck et al., 2008). The MFGM phospholipids not only contain phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol like those in soybean phospholipids, but also have a high level of sphingomyelin, which has been used as an alternative source of soybean phospholipids for food supplements (Astaire et al., 2003). On the other hand, MFGM phospholipids tend to be more saturated than soybean phospholipids because of the presence of a higher degree of saturation of fatty acid chains in the MFGM phospholipid molecules. Studies have shown that MFGM phospholipids can also be employed to prepare liposomes (Thompson et al., 2006; Thompson and Singh, 2006; Thompson et al., 2007). Compared with conventional soybean-derived materials, MFGM

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[1]These authors contributed to the work equally.
[2]Corresponding author: jgjiang@scut.edu.cn

phospholipids, with unique compositions and lower cost, may have broader application prospects.

Some studies have reported curcumin liposomes encapsulated by traditional or synthetic lecithin (Began et al., 1999; Takahashi et al., 2009; Patra et al., 2013), but the curcumin liposomes prepared with MFGM phospholipids have not been investigated. The objectives of the present study, respectively preparing curcumin liposomes with MFGM phospholipids and soybean lecithin, were to examine the ability of MFGM phospholipids to encapsulate curcumin by comparing the characteristics and stability of MFGM liposomes with those prepared with soybean lecithin. The processing parameters, size distribution, ζ-potential, and in vitro release of the 2 liposomes were investigated and determined. Their stability under various conditions, including pH, metal irons, light, temperature, oxygen, and relative humidity, was evaluated.

MATERIALS AND METHODS

Materials and Apparatus

The following materials were used in this study: curcumin (Aladdin Industrial Co., Ltd., Shanghai, China), MFGM phospholipids (prepared and preserved by our laboratory), soybean lecithin (Aladdin Industrial Co., Ltd., Shanghai, China), UV-Vis spectrophotometer [UV-2102PC, UNICO (Shanghai) Instruments Co., Ltd., China], Zetasizer Nano ZS (Malvern Instruments Co., Ltd., Malvern, UK), and pH meter (S-3D, Shanghai Precision and Scientific Instrument Co., Ltd., Shanghai City, China).

Preparation of Curcumin Liposomes

To compare the MFGM phospholipids with traditional membrane materials, the curcumin liposomes were prepared with MFGM phospholipids and soybean lecithin, respectively. The thin film ultrasonic dispersion method, a general procedure to obtain MFGM (Vermette et al., 2002) from fresh buffalo milk, was performed to prepare the liposomes. The MFGM phospholipids/soybean lecithin was dispersed in chloroform. Curcumin was dissolved in anhydrous ethanol and thoroughly mixed with the MFGM phospholipids/soybean lecithin dispersion in a 250-mL round-bottomed flask. The mixture solution was evaporated on a rotary evaporator at 35°C, until a thin film was formed on the flask. The film was washed with PBS and completely hydrated for 2 h. After the hydration of thin lipid film with PBS, the lipid dispersion was sonicated for 4 min, and finally the liposomes encapsulated with curcumin were obtained.

Determination of Curcumin Content and Encapsulation Efficiency

Precisely weighed curcumin was dissolved and diluted with anhydrous ethanol to form 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 2.0, 2.4, 2.8, 3.2 μg/mL of standard solution, the absorbance of which was measured at 424 nm with anhydrous ethanol as the blank. The linear regression analysis of absorbance on concentration was conducted and the regression equation was obtained. The regression equation of the standard curve was $y = 0.1718x + 0.003$, $R^2 = 0.999$. Every sample was diluted with anhydrous ethanol to a certain concentration, measured its absorption at 424 nm, and calculated the curcumin content according to the regression equation.

Encapsulation efficiency is a significant indicator in the quality evaluation of liposomes (Xia et al., 2012). The encapsulation efficiency (**EE**) was determined as follows:

$$\mathrm{EE} = \frac{m_1 - m_2}{m_1} \times 100\%,$$

where m_1 is the amount of total curcumin and m_2 is the amount of free curcumin in the liposome solution. Separation of liposome-incorporated and free curcumin was accomplished by centrifugation at 15,000 × *g* for 30 min at 4°C.

Investigation of Processing Parameters

The assessment of processing parameters was performed to analyze their influences on the encapsulation efficiency and determine the optimal conditions for preparing curcumin liposomes. The effects of ratio of curcumin to phospholipids/lecithin, ultrasonic time, PBS pH, concentration of PBS, and volume of PBS on the encapsulation efficiency were investigated in this study. The individual conditions of the 5 parameters were carried out as follows: ratio of curcumin to MFGM phospholipids 1:30, 1:35, 1:40, 1:45, 1:50; ratio of curcumin to soybean lecithin 1:10, 1:15, 1:20, 1:25, 1:30; ultrasonic time 1, 2, 3, 4, 5 min; PBS pH 5.0, 5.5, 6.0, 6.5, 7.0; concentration of PBS 0.005, 0.010, 0.020, 0.040, 0.060 mol/L; and volume of PBS 10, 15, 20, 25, 30 mL.

Measurement of Size Distribution and ζ-potential

Prior to the measurement of size distribution and ζ-potential, the liposome dispersions were diluted to the required turbidity with PBS solution. The average size and size distribution of curcumin liposomes were