LWT - Food Science and Technology 84 (2017) 34–46

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Preparation of curcumin-loaded emulsion using high pressure homogenization: Impact of oil phase and concentration on physicochemical stability

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ARTICLE INFO

Article history:
Received 15 October 2016
Received in revised form
24 April 2017
Accepted 25 April 2017
Available online 27 April 2017

Keywords:
Curcumin
Nanoemulsion
Microstructure
Storage stability

ABSTRACT

Curcumin is a natural food coloring agent with many pharmacological effects such as anti-oxidation and anti-cancer. In this study, curcumin was dissolved in different oil phases (medium chain triglyceride, canola oil and linseed oil) by different treatments (heat, ultrasonic and microwave). Curcumin nanoemulsions stabilized by different emulsifiers (Tween-80, lecithin, whey protein isolation and acacia) with different concentrations were prepared using high pressure homogenization to evaluate physicochemical properties including curcumin content, particle size, potential, physical stability and storage stability (variation of curcumin content and particle size under different temperatures in 60 days). The increase in oil phase concentration caused increase in curcumin content, particle size and viscosity of the emulsions but decrease in stability. Meanwhile, temperature significantly affected the stability of nanoemulsion stabilized by lecithin instead of Tween-80. Curcumin nanoemulsion could achieve the maximum curcumin content when choosing medium chain triglyceride as its oil phase. These results may broaden the application of curcumin in food industry for improving its solubility and bioavailability.

1. Introduction

As the natural food coloring agent, curcumin was a kind of polyphenols extracted from rhizomes of *Curcuma longa*. Recent studies have found that curcumin had a wide range of pharmacological effects, such as anti-oxidation, anti-inflammatory (Kaur & Das, 2011), antibacterial (De et al., 2009), anticancer (Fang, Lu, & Holmgren, 2005; Li et al., 2014) and treatment of diabetes (Bengmark, 2006). Due to the poor stability under light, heat and iron ion conditions, the use of curcumin was limited in food manufacturing. The water-soluble fraction of curcumin lacks stability as well (Manju & Sreenivasan, 2011). This challenge could be overcomed by developing delivery systems such as nanoemulsion, and bioactive compounds could be better preserved, which will facilitate their application in industry.

Recent study has shown that an increase in stability, water solubility, antioxidant and antibacterial activities was observed in encapsulated curcumin (Zhao, Pan, Nitin, & Tikekar, 2014). The stability of the emulsion system is subjected to the pH, the type of functional factors, particle size, surface charge, fluidity and so on (Waraho, Cardenia, Decker, & McClements, 2010). According to the study, under a pH between 4 and 7, although aggregation occurred among a large number of particles, nanoparticles remained a relatively small particle size in the absence of neutral salt. Under the same environmental conditions, different types of emulsifiers also affected the stability of the carrier system. Besides, ingredients such as pro-oxygenic agent and antioxidant existed on the oil-water interface of emulsion had an important effect on the stability of the delivery system (Frankel, Huang, Kanner, & German, 1994; McClements & Decker, 2000).

There are many different methods or systems was used to encapsulate curcumin, including self-assembly (Yallapu, Jaggi, & Chauhan, 2012) nanocurcumin (Bisht et al., 2007; Esmaili et al., 2011), liposomes (Li, Ahmed, Mehta, & Kurzrock, 2007), phospholipid mixture (Maiti, Mukherjee, Gantait, Saha, & Mukherjee, 2007). Futhermore, curcumin-metal chelate (John, Kuttan, &

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http://dx.doi.org/10.1016/j.lwt.2017.04.074
0023-6438/

Krishnankutty, 2002) has been employed to protect its biological activity effectively. And more importantly, the time that the encapsulated curcumin in the serum was significantly increased compared with natural curcumin (Anand et al., 2010). Also the anti-inflammation of curcumin was enhanced when using polylactic acid-glycolic acid to embed curcumin (Thamake, Raut, Gryczynski, Ranjan, & Vishwanatha, 2012). To our knowledge, there was no research that previously examined the influence of food-grade oil phase and concentrations on physicochemical properties and stability of curcumin loaded emulsion system. This study aimed to prepare the emulsion with high content of curcumin and a good stability, providing reference for improving the application of curcumin in food industry.

2. Materials and methods

2.1. Materials and chemicals

Curcumin (95% purity, Tianxu Biological Technology Co., Ltd, Hebei, China), medium-chain triglyceride oil (MCT, Guangdong Wincom Flavors & Fragrances Co., Ltd, China), canola oil (Canola, CAN), linseed oil and sunflower oil (Jinye ASTCo., Ltd), corn oil (Sanxing Corn industry science and technology co., LTD), lecithin (food grade, De city food products factory, He'nan, China), Acacia senegal (TIC GUMS, China), Tween-80 (Xilong Chemical industry Company Limited, China), ethanol (every 100 g contains 95 g of ethanol), deionized water.

2.2. Solubility of curcumin in various oils

Five different oils phases (canola oil, sunflower oil, linseed oil, corn oil and MCT) and three different dissolution methods, including stirring in boiling bath for 3 min, ultrasonic (JY32-11N Ultrasonic homogenizer; Ningbo Scientz Biotechnology Co. Ltd) in the conditions of one second interval and 390 W for 30 min and microwave treatment (780W for 30s) were chosen to investigate the solubility of curcumin. Briefly, 1.5 g of curcumin was dissolved into five different oils by three above-mentioned methods respectively and mixtures were kept at room temperature for 24 h. Then mixtures were centrifuged ($531\times g$ for 2 min) and supernatant was collected to determine the absorbance by UV spectrophotometer (Shimadzu Corporation, Kyoto, Japan) at 425 nm. The content of curcumin in different oil phases was determined according to the standard curve. First, dissolving 6 mg of curcumin in 100 mL of 95% ethanol, and 0.25 mL, 0.5 mL, 1 mL, 2 mL, 3 mL and 4 mL of solution were taken and diluted to 15 mL with alcohol respectively (curcumin concentration from 1 mg/L to 16 mg/L and use 95% ethanol as blank). To set up the standard curve of curcumin content, absorbance as Y-axis was plotted against curcumin concentration on X-axis ($Y = 0.0146X+0.0222$, $R^2 = 0.9993$). All determinations were performed in triplicate.

2.3. Preparation of curcumin-encapsulated emulsions

Oil-in-water emulsions of curcumin were prepared by high pressure homogenization. Four different emulsifiers (Tween-80, lecithin, acacia senegal and whey protein) were applied to stabilize emulsions. Tween-80 (20 g), lecithin (50 g), acacia powder (50 g) and whey protein (50 g) was dissolved and diluted to 1 L by deionized water severally to be the stock solution before magnetic stirred for 4 h. 3 g of curcumin was added into 250 mL of oils (three kinds of oils included MCT, linseed oil and canola oil), and treat them with ultrasonication. After centrifugation, the supernatant was collected as oil phase. The stock solution and the supernatant was kept in dark at room temperature for 24 h. Different weights of supernatant (7.5 g, 15.0 g, 22.5 g, 30.0 g, 37.5 g, 45.0 g) were added into the stock solution and the total weight of mixtures was fixed (150 g). After that, mixtures were sheared to form a coarse emulsion by high-speed blender (Ultra-Turrax T25, IKA, Staufen, Germany) at 10000 rpm for 6 min. The homogenization method was according to the previous study with some modification (Liu, Wang, Sun, McClements, & Gao, 2016). Finally, the obtained coarse emulsions were homogenized at 60 MPa using a high pressure homogenizer (Type NS 1001L2K, GEA Niro Soavi S.p.A, Parma, Italy) for three cycles to form fine emulsion. All samples were prepared in triplicate.

2.4. Particle characteristics of curcumin loaded emulsions

The content of curcumin in the nanoemulsion were determined by the UV spectrophotometer at a wavelength of 425 nm at room temperature after diluting 1000 times in 95% ethanol. The particle hydrodynamic diameter and zeta-potential were determined by dynamic light scattering (DLS) using Malvern Zetasizer Nano ZS90 (Malvern Instruments, Worcestershire, UK) (Abbas, Bashari, Akhtar, Li, & Zhang, 2014). Particle size determination was conducted by diluting the emulsions with deionized water to 500-fold, which was to avoid the multiple light scattering and the impact on the accuracy of measurement. The similar dilution procedure was conducted in zeta-potential measurement, the difference was that samples were diluted with 95% ethanol to 1000-fold. All determinations were performed in triplicate at 25 °C after 120 s of equilibration.

2.5. Determination of physical stability

The physical stability of the curcumin loaded emulsion was analyzed with LUMiSizer (L.U.M. GmbH, Berlin, Germany), an instrument employing centrifugal force to accelerate the occurrence of instability phenomena such as sedimentation, flocculation, or creaming (Tan, Feng, Zhang, Xia, & Xia, 2016). Emulsion stability was shown as a space-and-time-related transmission profile over the sample length. The instrumental parameters used for the measurement were as follows: volume, 1.8 mL of dispersion; 4000 rpm; time Experiment, 127.5 min; time interval, 30 s; temperature, 25 °C.

2.6. Cryo-scanning electron microscopy (Cryo-SEM)

Morphology of the emulsions was observed by SEM (JEOL, JSM-6701F, Japan) at an accelerating voltage of 10.0 kV. Prior to the observation, a drop of sample was dripped on silicon slice followed by sputter-coated with a gold layer to avoid charging under the electron beam.

2.7. Determination of storage stability

After preparation of the emulsions, the samples were deposited at 4 °C, 25 °C and 55 °C for 60 days. At selected time points, the curcumin content and particle size were determined to represent the change of its characteristics. All determinations were performed in triplicate.

2.8. Statistical analysis

All data were performed in triplicate and values were expressed as the average and standard deviation. Data were analyzed by one-way analysis of variance using the SPSS 16.0 package (SPSS Inc., Chicago, USA).