Food Hydrocolloids 44 (2015) 220–228

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Design and fabrication of a food-grade albumin-stabilized nanoemulsion

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ARTICLE INFO

Article history:
Received 2 April 2014
Accepted 3 September 2014
Available online 19 September 2014

Keywords:
Nanoemulsion
Ultrasonication
Delivery system
Bovine serum albumin
Polyethylene glycol

ABSTRACT

A food-grade biopolymer-emulsion delivery system was developed for improving or extending the functional performance of the bioactive components of food. Droplet size, rheological and interfacial measurements, scanning electron microscopy (SEM), thiobarbituric acid reactive substance (TBARS) assay and Fourier transform-infrared (FTIR) analysis were used to study the effect of sonication time and power, poly ethylene glycol (PEG) addition, heat treatment and oil fraction on physical and chemical stabilities of bovine serum albumin (BSA)-stabilized corn oil-in-water (O/W) emulsions prepared by ultrasonic emulsification. The findings showed that sonication power and time had significant effects on the reduction of mean droplet diameter to 75 nm in the presence of PEG. PEG acted as an enhancer of viscosity of the aqueous phase. PEG had negligible influence on the interfacial tension of the emulsion. An increase in the oil fraction of up to 20 wt% had no effect on the mean droplet diameter of BSA emulsion containing PEG. Mean droplet diameter of BSA emulsions that contained PEG 10000 and 300, increased to 170 and 250 nm, respectively, after 60 days of storage at 6 °C. FTIR analysis showed that the addition of PEG had no major influence on the secondary structure of BSA. The proportion of unadsorbed protein in the PEG-contained nanoemulsions was lower than that in emulsions without PEG. Based on TBARS content the oxidative stability of sonicated emulsions in the presence of PEG were less than other formulations.

1. Introduction

In recent years, a growing interest has been raised in the delivery of a wide range of bioactive components in the food and pharmaceutical industries using food protein stabilized emulsions. These proteins have a great potential as a safe stabilizer for oil-in-water (O/W) emulsions, good binding capacity for hydrophobic molecules and also excellent gelation properties that facilitate their use for means of delivery (McClements, Decker, Park, & Weiss, 2009; McClements, Decker, & Weiss, 2007). On the other hand, many nanoparticle formulations are based on a nanoemulsion template (Anton, Benoit, & Saulnier, 2008; Jones & McClements, 2010). Molecular flexibility and surface

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http://dx.doi.org/10.1016/j.foodhyd.2014.09.005
0268-005X/

hydrophobicity are two important properties for the quality determination of proteins used as emulsion stabilizers (Grigoriev & Miller, 2009). Bovine serum albumin (BSA) is known as one of these qualified protein stabilizers. BSA is the most abundant protein in blood plasma with high solubility and stability available at high purity and low cost. BSA has been confirmed to show good emulsifying properties with an emulsifying ability superior to that of soy proteins, β- or κ-casein, ovalbumin and β-lactoglobulin (Tang & Shen, 2013). It has excellent gelation properties by heat and cold set methods (Navarra et al., 2009), which would be an important characteristic in the preparation of nanoparticles. BSA can form a shield by adsorbing to the O/W interface, binding, entrapping or coating a bioactive molecule (Kratz, 2008; Livney, 2010).

Nanoemulsions are metastable dispersions of sub-100 nm droplets of a liquid in a different immiscible liquid (Fryd & Mason, 2012). Nanoemulaions have many potential advantages over conventional emulsions for particular applications in food and beverage products, Some of these advantages include high optical clarity, good physical stability and enhanced bioavailability, but there may also be some risks such as the ability to change the biological fate of bioactive components within the gastrointestinal tract (Acosta, 2009; Mason, Wilking, Meleson, Chang, & Graves, 2006). A high-energy input is generally needed to produce nanoemulsions. Using ultrasound as a high-energy source has numerous advantages such as lower energy consumption, less surfactant use and smaller droplet size. More homogenous batches are usually achieved compared with conventional mechanical processes (Abismaıl, Canselier, Wilhelm, Delmas, & Gourdon, 1999). The use of sonotrode to generate sound waves with frequency between 20 and 24 kHz can cause mechanical vibrations that lead to the development of acoustic cavitation. These cavities collapse and generate powerful shock waves that break microdroplets into nanodroplets (Abismaıl et al., 1999; Canselier, Delmas, Wilhelm, & Abismail, 2002; Hielscher, 2007). Droplet size and distribution in ultrasound emulsification can be controlled by optimizing various parameters such as energy input (power), nature and amount of emulsifiers, type of oil and emulsification time. Other parameters such as continuous phase viscosity and volume fraction of oil also affects the formation of nanoscale droplets (Behrend, Ax, & Schubert, 2000; Delmas et al., 2011; Gaikwad & Pandit, 2008). The ability to initially create an emulsion with nanoscale droplet sizes and subsequent stabilization against Ostwald ripening are two important challenges in the formulation of nanoemulsions (Wooster, Golding, & Sanguansri, 2008).

Despite the great potential of nanoemulsions in fundamental and applied sciences, only a few studies have been carried out on food grade nanoemulsion formation, ranging from 50 to 100 nm (Delmas et al., 2011; He et al., 2011; Kentish et al., 2008; Rao & McClements, 2012). No report has been published on BSA-based nanoemulsion using high viscosity oils such as long chain triglycerides and high molecular weight proteins as emulsifiers. Therefore, the possibility of the formation of a stable BSA-based nanoemulsion for delivery of bioactive compounds was investigated in this study. Furthermore, the influence of sonication power and time, addition of high or low molecular weight poly ethylene glycol (PEG) in aqueous phase, heat treatment of BSA solution, oil fraction on mean droplet size and its physical and oxidative stability have been investigated in O/W emulsions prepared by ultrasound emulsification. Moreover, the effect of PEG on protein structure was evaluated by Fourier transform-infrared (FTIR) spectroscopy analysis. Unadsorbed proteins were also measured.

2. Materials and methods

2.1. Materials

Lyophilized bovine serum albumin was purchased from Sigma–Aldrich Chemical Company, USA, and stored in the refrigerator at 4 °C. PEG (average molecular weights of 300, 10000) butylated hydroxytoluene (BHT), 1, 1, 3, 3-tetraethoxypropane, trichloroacetic acid (TCA), thiobarbituric acid (TBA) were also supplied by Sigma–Aldrich. Commercially available corn oil of the Kasisuri Company (Thailand) was purchased from the local supermarket and used as oil phase. Sterile double distilled water was used for sample preparation.

2.2. Nanoemulsion preparation

BSA solutions were prepared by dissolving of BSA powder into sterile double distilled water stirred to enable complete hydration. The aqueous phases of emulsion were prepared in four different formula (F) types: F1) 15 wt% BSA solution. F2) 15 wt% BSA + 20 wt% PEG300 solution. F3) 15 wt% BSA + 20 wt% PEG1000 solution, and F4) 15 wt% BSA heated at 58 °C for 2 h. The ratio of oil phase (corn oil) to aqueous phase was set at 5, 10 and 20%. PEG 300 and 10000 had no capability to form emulsion and the oil phase was separated from the aqueous phase immediately after emulsification.

A coarse emulsion was prepared by homogenization using high speed Ultra-Turrax blender (Hiedoloph, Germany) at 22,000 rpm for 5 min. The coarse emulsion was then further emulsified using 20 KHz ultrasonicator, UP200S and UP400S (Dr. Hielscher, Germany) with a maximum power output of 200 or 400 W. A preliminary study was carried out to optimize protein concentration. Energy input was given throughout a sonotrode H3 containing a piezoelectric crystal with a titanium probe diameter of 3 mm. The amplitude of oscillation was set at 100 microns. The temperature difference between the primary coarse emulsion and the final emulsion was less than 10 °C. Increase of temperature during ultrasonication was inhibited by placing the sample container in a bigger beaker containing ice. The volume of the coarse emulsion was set to 5 ml in all samples and the sonotrode was located 0.5 cm below the surface of the emulsion. The pH of the sample was checked by pH meter (Metrohm, Germany).

2.3. Particle size and Zeta potential measurements

The mean droplet diameter and size distribution of emulsions were determined by dynamic light scattering (DLS) using Nanotrac Wave® (Microtrac, USA). To avoid multiple scattering effect, all samples were diluted 250 folds in deionized water before measurement. A refractive index of 1.33 was used for BSA stabilized emulsions. Emulsion droplet size was reported as the mean diameter of volume distribution (MV):

$$MV = \frac{\Sigma vidi}{\Sigma vi} \quad (1)$$

where vi is the volume percent between droplet size and di is the diameter of droplets.

The physical stability of nanoemulsion was determined by measuring the mean droplet size of nanoemulsion every week during storage at 6 °C for 60 days. The surface charge of emulsion was measured using the same light scattering apparatus.