LWT - Food Science and Technology 76 (2017) 344–350

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Formulation of W/O/W emulsions loaded with short-chain fatty acid and their stability improvement by layer-by-layer deposition using dietary fibers

Yohei Yamanaka [a, b], Isao Kobayashi [b, *], Marcos A. Neves [a, b], Sosaku Ichikawa [a, b], Kunihiko Uemura [a], Mitsutoshi Nakajima [a, b]

[a] Food Research Institute, NARO, 2-1-12 Kannondai, Tsukuba, Ibaraki 305-8642, Japan
[b] Graduate School of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki 306-8572, Japan

ARTICLE INFO

Article history:
Received 22 February 2016
Received in revised form
28 July 2016
Accepted 28 July 2016
Available online 2 August 2016

Keywords:
W/O/W emulsion
Short-chain fatty acid
Layer-by-layer deposition
Dietary fiber
ζ-potential

ABSTRACT

This study prepared water-in-oil-in-water (W/O/W) emulsions loaded with short-chain fatty acid (SCFA) and attempts to improve their stability with layer-by-layer deposition using dietary fibers. The water-in-oil (W/O) emulsion consisted of an inner water phase containing 5 wt% butyric acid and soybean oil phase containing a hydrophobic emulsifier. The weight fraction of the inner water phase was 5 wt%. W/O emulsions with a Sauter mean droplet diameter ($d_{3,2}$) of 0.4 μm were prepared using high-pressure homogenization. The W/O/W emulsion consisted of W/O emulsion dispersed in an outer water phase containing 0.5 wt% modified lecithin, and the weight fraction of the W/O phase was 20 wt%. The W/O phase was dispersed in the modified lecithin solution to prepare a W/O/W emulsion using rotor-stator homogenization. The $d_{3,2}$ of the resultant W/O/W emulsion droplets was 16.9 μm, and their ζ-potential was −77.8 mV at pH 5. W/O/W emulsion droplets were coated with chitosan (CHI) and carboxymethyl cellulose (CMC), which is driven by electrostatic interaction. The ζ-potential data indicated successful coating of the droplets. W/O/W emulsion droplets coated with dietary fibers were highly stable over 4 weeks. Our results demonstrated that W/O/W emulsions loaded with SCFA could be better stabilized with layer-by-layer coating using dietary fibers.

1. Introduction

In general, emulsion is defined as the dispersion of two immiscible fluids (e.g., oil and water) into one another as small spherical particles. The droplet size of emulsions prepared using different techniques is generally 0.1–100 μm (Clausse, Gomez, Dalmazzone, & Noik, 2005; McClements, 2004). A rotor-stator homogenizer is frequently used in food industries to prepare nonuniform droplets of 2–10 μm. A high-pressure homogenizer can produce droplets having a monomodal size distribution on a submicron scale (McClements, 2004). Emulsions are a thermodynamically unstable but semi-stable systems (Okazawa & Bron, 1979). If an emulsion does not contain an emulsifier and a stabilizer, phases eventually separate. An emulsifier and a stabilizer are required to improve emulsion stability, which is greatly influenced by the viscosity and density of liquid phase and ζ-potential of droplets. Viscosity and density also influence creaming/sedimentation of the droplets, which can be expressed by Stokes' law. Droplets with high absolute ζ-potential are capable of preventing aggregation, due to electrostatic repulsion.

There is a wide variety of food emulsions. Oil-in-water (O/W) food emulsions include mayonnaise, dressing, milk, and soups. Water-in-oil (W/O) food emulsions include butter, margarine, and chocolates. Multiple emulsions, known as “emulsions of emulsions,” may be water-in-oil-in-water (W/O/W) emulsions consisting of oil droplets containing smaller droplets of an inner water phase dispersed in water phase (Aserin, 2007). Emulsions containing functional ingredients have received a great deal of attention in food industries (McClements & Li, 2010a, 2010b). Hydrophilic functional ingredients can be encapsulated in W/O

* Corresponding author. Food Engineering Division, National Food Research Institute, NARO, 2-1-12 Kannondai, Tsukuba, Ibaraki 305-8642, Japan. Tel.: +81 29 838 8025; fax: +81 29 838 8122.
E-mail addresses: isaok@affrc.go.jp (I. Kobayashi), nakajima.m.fu@u.tsukuba.ac.jp (M. Nakajima).

http://dx.doi.org/10.1016/j.lwt.2016.07.063

emulsions, and hydrophobic functional ingredients can be encapsulated in O/W emulsions. W/O/W emulsions can load both hydrophilic and hydrophobic functional ingredients (Aserin, 2007; Farahmand, Tajerzadeh, & Farboud, 2006; Kanafusa, Chu, & Nakajima, 2007; Lee et al., 2004; Khalid, Kobayashi, Neves, Uemura, & Nakajima, 2013; Khalid et al., 2014; and Trentin, DeLamo, Guell, Lopes, & Ferrando, 2011).

Short-chain fatty acids (SCFA) are water-soluble fatty acids with a carbon number lower than 6. SCFA is an important energy source for intestinal epithelial cells. For example, butyric acid represents a major energy supply for the large intestine. SCFA prevent colon carcinogenesis (Hamer et al., 2008) and help maintain the colonic environment (Greer & O'Keefe, 2011). Large intestine utilizes SCFA that were produced in proximal colon as its energy source (Cummings, Rombeau, & Sakata, 1995). Several foods and supplements are rich in enteric bacteria and dietary fiber. However, the enteral environment influences the type and quantity of SCFA (Cummings et al., 1995). Therefore, delivering SCFA directly to the large intestine effectively improves the enteral environment. SCFA have an unpleasant smell, so that endeavoring their oral intake. To solve this problem, Li and co-workers obtained O/W emulsion loaded with tributyrin as the carrier of butyric acid (Li, Maux, Xiao, & McClements, 2009). Tributyrin is lipophilic and has major characteristics different from those of SCFA. Since SCFA are water-soluble, the solution containing SCFA should be used as the inner water phase of W/O or W/O/W emulsion. W/O/W emulsion is a potential carrier of SCFA to the large intestine, whereas the oil phase of W/O/W emulsion is digested by lipase in the stomach and small intestine (McClements & Li, 2010a, 2010b). SCFA absorbed in the small intestine cannot reach the large intestine. Therefore, it is necessary to improve stability of these W/O/W emulsions in the gastrointestinal tract. Dietary fibers are normally not digested in the gastrointestinal tract, since they are decomposed by enteric bacteria (Cummings et al., 1995).

Layer-by-layer deposition is one method of multicoating using electrostatic polymers to the object (Richardson, Bjornmalm, & Crauso, 2015). Dietary fibers that have electrical charge such as chitosan (CHI) and carboxymethyl cellulose (CMC) can be used for layer-by-layer deposition. W/O/W emulsion coated with dietary fiber may increase the physical stability and reach the large intestine without being digested. Layer-by-layer deposition has been applied to only O/W emulsions thus far (Bortnowska, 2015; Iwata, Neves, Watanabe, Sato, & Ichikawa, 2014).

The purposes of this study are to formulate W/O/W emulsions coated with dietary fibers using layer-by-layer deposition as a potential carrier of SCFA to the large intestine and to evaluate their physical stability in terms of coalescence stability of W/O/W emulsion droplets. Butyric acid was selected as the model SCFA in this study.

2. Materials and methods

2.1. Materials

The SCFA used in this study was butyric acid purchased from Sigma-Aldrich Co. (St.Louis, USA). Refined soybean oil was provided by Wako Pure Chemical Industries Ltd. (Osaka, Japan) as the oil phase of the W/O emulsion. Tetra glycerin condensation ricinoleate (TGCR, HLB<1) was provided by Sakamoto Yakuhin Kogyo Co., Ltd. (Osaka, Japan) as the emulsifier of W/O emulsion. Modified lecithin (SLP WhiteLyso) was provided by Tsuji Oil Mills Co., Ltd. (Matsuzaka, Japan) as the emulsifier of W/O/W emulsion. The modified lecithin consists of phosphorus lipid content of consists of lysophosphatidylcholine (18–30%), phosphatidylinositol (10–20%), phosphatidylcholine (2–8%), phosphatidylethanolamine (1–7%) and phosphatidic acid (0–5%). The critical micellar concentration of the modified lecithin was 0.065 wt% (Fig. S2). CHI (CHI HD; molecular weight 58 kDa; degree of deacetylation of 98%), was provided by Yaegaki Bio-industry, Inc. (Himeji, Japan). CMC was provided by Wako Pure Chemical Industries, Ltd. CHI and CMC were used as the dietary fibers for layer-by-layer deposition.

Acetic acid, sodium acetate, hydrochloric acid (1 mol/L), chloroform, hexane, and sodium hydroxide (1 mol/L) were purchased from Wako Pure Chemical Industries, Ltd. Fluorescein isothiocyanate (FITC) was provided by Sigma-Aldrich Co.

2.2. Emulsion preparation

2.2.1. Solution preparation

Water solutions containing butyric acid as the inner water phase of W/O emulsion were prepared by dissolving 1, 3, and 5 wt% butyric acid in Milli-Q water. Soybean oil solution containing 5 wt% TGCR was used as the oil phase of W/O emulsion. Modified lecithin solution was prepared as the outer water phase of W/O/W emulsion by dissolving 0.5 wt% modified lecithin in Milli-Q water. An acetate buffer solution (pH5) was prepared by solubilizing sodium acetate into acetic acid (30 mmol/L) used for diluting W/O/W emulsion samples.

2.2.2. Preparation of W/O emulsion loaded with SCFA

The W/O emulsions were prepared by dispersing an internal water phase (5 wt%) into an oil phase (95 wt%) by using a rotor-stator homogenizer (Polytron PT-3100, Kinematica Co., Ltd., Luzern, Switzerland.) at 10,000 rpm for 5 min, followed by a high-pressure homogenizer (NanoVater200, Yoshida Kikai Co., Ltd., Nagoya, Japan) at 100 MPa for one pass.

2.2.3. Preparation of W/O/W emulsion

The W/O/W emulsion was prepared by dispersing a W/O emulsion (20 wt%) into an outer phase (80 wt%) that is an aqueous solution containing 0.5 wt% modified lecithin by using rotor-stator homogenization at 5,000 rpm for 5 min. The pH of the primary W/O/W emulsion was adding 200 mL of an acetate buffer solution in 50 mL of this emulsion sample.

2.3. Coating of W/O/W emulsion droplets by layer-by-layer deposition using dietary fibers

2.3.1. Solution preparation

An aqueous phase containing CHI was prepared by dissolving 1 wt% mixture of CHI powder (90 wt% pure-CHI powder and 10 wt% FITC-labeled CHI powder (Huang, Ma, Khor, & Lim, 2002)) in Milli-Q water. Hydrochloric acid (1 mol/L) was added to dissolve the CHI powder in this aqueous phase at pH 2. Afterwards, sodium hydroxide (1 mol/L) was added to adjust the pH of the CHI-containing solution to 5. The ionic strength of the CHI-containing solution (pH 5) is 1.0×10^{-5} mol/L, and that of the W/O/W emulsion mixed with CHI-containing solution is 2.9×10^{-6} mol/L. These ionic strength values may not affect the properties of the W/O/W emulsion coated with CHI. The CMC was prepared by dissolving ca. 1 wt% CMC powder in the acetate buffer solution.