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Encapsulation of β-sitosterol plus γ-oryzanol in O/W emulsions: Formulation characteristics and stability evaluation with microchannel emulsification

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ABSTRACT

β-Sitosterol and γ-oryzanol have reduced solubility in aqueous based formulations. In this study β-sitosterol (β-st) and γ-oryzanol (γ-oz) were encapsulated at relatively high concentrations in different food-grade oil-in-water (O/W) emulsions using straight-through microchannel emulsification. The innovative aspect of this study was the production of monodisperse droplets with high encapsulation efficiency and stability of β-sitosterol and γ-oryzanol. Milli-Q water containing 1% (w/w) Tween 20 or decaglycerol monolaurate (ML-750) was used as the continuous phase and the dispersed phase contained 0.5–4% (w/w) each of β-st and γ-oz in medium chain triglycerides. Successful droplet generation was conducted with different concentrations of β-st and γ-oz. The Sauter mean diameter of 1% (w/w) β-st and γ-oz loaded O/W emulsions ranged between 26 and 28 μm with relative span factor width below 0.21. These emulsions were stable at 4 and 25 °C during evaluated storage period. The emulsions stabilized with Tween 20 have encapsulation efficiencies of β-st and γ-oz ($EE_{β\text{-st}}$ and $EE_{γ\text{-oz}}$) above 80% at 4 and 25 °C; those stabilized with ML-750 have $EE_{β\text{-st}}$ over 80% and $EE_{γ\text{-oz}}$ above 50% at 4 and 25 °C.

1. Introduction

An emulsion is a mixture of two immiscible liquids containing one liquid dispersed in the form of small droplets in another liquid that forms a continuous phase. These different phases constitute oil-in-water (O/W) or water-in-oil (W/O) emulsions like milk or butter (Schramm, 2006). The emulsions play extremely important roles in different applications like food processing (Leal-Calderon et al., 2007), oil recovery (Huang and Varadaraj, 1996), toxic material handling (Ouyang et al., 1995) and different drug deliveries (Nakano, 2000). The emulsions are stabilized by using different emulsifiers and these emulsifiers migrates to the liquid–liquid interface and inhibit droplet coalescence and flocculation (Schramm, 2006).

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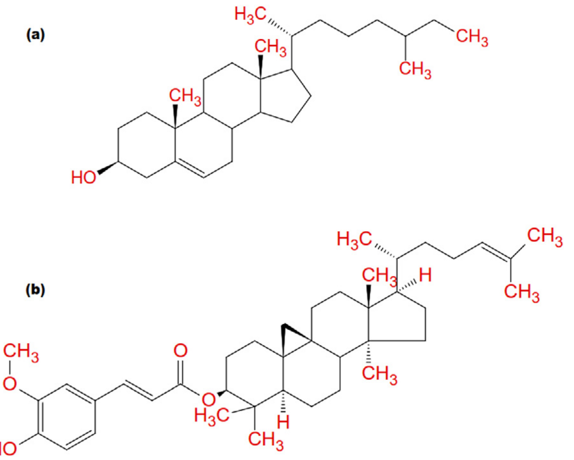

Fig. 1 – Structural representation of bioactives. (a) β-st; (b) γ-oz.

Conventional methods for making emulsions involve droplet breakup using high energy shear forces together with elongation and cavitation forces. These forces are not uniform entirely in emulsion system and contribute polydispersity in system (Santana et al., 2013). Moreover, these high shear, elongation and cavitation forces have low energetic efficiencies and low encapsulation efficiencies (Santana et al., 2013). Modern emulsification methods like microthread generation (Gañán-Calvo, 1998), viscoelastic shear (Perrin, 1998), membrane emulsification (Nakashima et al., 1991) and microchannel emulsification (MCE) (Kawakatsu et al., 1997) have been developed and optimized for better control over droplet size and system properties (Schroen et al., 2015). These methods are more energy efficient and have better encapsulation efficiencies over time. The other attractive feature of microfluidic devices is fabrication of double, triple or even higher order emulsions with extreme monodispersity and unprecedented accuracy (Chu et al., 2007; Utada et al., 2005).

In this study, we use the striking features of MCE to encapsulate γ-oryzanol (γ-oz) together with β-sitosterol (β-st) in O/W emulsions. Previous MCE studies describe the encapsulation of single bioactive like vitamin D (Khalid et al., 2015a,b), ascorbic acid derivatives (Khalid et al., 2014a), L-ascorbic acid (Khalid et al., 2014b), polyunsaturated fatty acids (Neves et al., 2008a) and oleuropein (Souilem et al., 2014). However, there is no MCE study that shows the droplet generation behavior with multiple nutrients and their storage stability. The present study explores the possibility of multi-nutrients encapsulation with MCE. The term MCE was coined by Kawakatsu et al. (1997) and the MCE system has capacity to form monodisperse emulsion droplets using micro-fabricated microchannel (MCs) on a silicon microchip. These MC arrays can be fabricated as microgrooves horizontally to the microchip surface (Chuah et al., 2009) or vertically as straight-through microholes (Kobayashi et al., 2005a). The grooved MC array chips exhibit low droplet productivity due to limited number of MCs but are extremely productive in elucidating droplet generation behavior from MCs. The straight-thorough microchips consist of a maximum of several hundreds of thousands of MCs (Kobayashi et al., 2005b) and have monodisperse droplet productivity even at dripping regime of 90 mL h^{-1} (Vladisavljevic et al., 2011). The mechanism of droplet formation in MCE was recently reviewed by Vladisavljevic et al. (2013) and Vladisavljević et al. (2012). Monodisperse emulsions with droplet diameters of 1 μm–500 μm and the smallest coefficient of variation below 5% have been successfully formulated through MCE (Vladisavljević et al., 2012).

γ-Oz (Fig. 1a) is a functional compound present in the rice germ oil and bran which contains triterpene alcohols and mixture of ferulic acid esters (Patel and Naik, 2004). γ-Oz has strong antioxidant and lipid peroxidation inhibition effects. Kanno et al. (1985) reported that γ-oz (0.5–1%) inhibited thermal oxidative polymerization of soybean oil. Wilson et al. (2007) reported that γ-oz reduced plasma cholesterol in hypercholesterolemic hamsters. γ-Oz also plays it role in treatment of relieving menopausal symptom (Murase and Iishima, 1963). β-St (Fig. 1b) is a predominant phytosterol found in higher plants as well as in human foods (Phillips et al., 2005). β-St is the most extensively studied phytosterol due to its role in hypercholesterolemia (Scholle et al., 2009), cardiovascular diseases (Genser et al., 2012) and benign prostatic hyperplasia (Berges et al., 2000). β-St is used in a variety of enriched commercial foods such as fruits juice, milk, yoghurt and spreads. Safety concerns regarding the use of β-st have been well addressed in different in vivo and clinical studies (Hamedi et al., 2014; Katan et al., 2003).

There is a considerable interest in structuring, fortifying and supplementing foods, oils and beverages with plant based phytochemicals. The difficulties behind phytosterol encapsulation include hydrophobicity in food matrixes (Ghaderi et al., 2014), degradability at high temperatures (Khuwijitjaru et al., 2009), low water solubility (Delaney et al., 2004) and moisture contents (Gawrysiak-Witulska et al., 2012). In this study, we formulate monodisperse O/W emulsions containing mixture of γ-oz and β-st. Moreover, we investigated the effect of different concentrations of γ-oz and β-st on droplet formation characteristics. The physical and chemical stability of monodispersed O/W emulsions stabilized by two different emulsifiers were also investigated.

2. Materials and methods

2.1. Chemicals

γ-Oryzanol, ethyl acetate, chloroform, acetic anhydride, sulfuric acid and polyoxyethylene (20) sorbitan monolaurate (Tween 20) were purchased from Wako Pure Chemical Ind. (Osaka, Japan). Medium-chain triglyceride oil (MCT, sunsoft MCT-7) with a fatty acid residue composition of 75% caprylic acid and 25% capric acid was procured from Taiyo Kagaku Co. Ltd. (Yokkaichi, Japan). β-Sitosterol was purchased from MP biomedicals (Illkirch, France). Decaglycerol monolaurate (ML-750, HLB 14.8) was kindly provided by Sakamoto Yakuhin Kogyo Co., Ltd. (Osaka, Japan). Milli-Q water having resistivity of 18 MΩ cm and pH of 7.1 was served as the continuous phase.

2.2. Asymmetric microchannel array chip

The emulsification experiments were conducted using 24 × 24 mm silicon MC array chip (Model WMS 1-4, EP. Tech Co., Ltd., Hitachi, Japan) containing 23,348 MCs and four 2.0 mm diameter holes at the corners of the plate. These holes were used to feed both phases and collect the produced emulsions. These MCs were micro-fabricated using repeated process of photolithography and deep-reactive-ion etching (DRIE) and located within 10 × 10 mm square region in the center of plate. The MC array chip was 500 μm thick (Fig. 2a), but was etched down to thickness of 100 μm in the central region where MCs were located. Each MC consist of cylinderal microhole of 10 μm with 70 μm depth and a microslot (10 × 50 μm cross section and 30 μm depth) (Fig. 2c and d). The distance between the centers of adjacent MCs in the vertical rows was 70 μm and the distance between the centers of MCs in the adjacent rows was 60 μm (Fig. 2d). The MC array chip was plasma oxidized to grow hydrophilic silicondioxide layer using an oxygen plasma reactor (PR500, Yamato Science Co. Ltd., Tokyo, Japan).

2.3. Formulation of continuous and dispersed phases

The continuous phase was formulated in Milli-Q water using either 1% (w/w) ML-750 or 1% (w/w) Tween 20 as emulsifiers.