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Microchannel emulsification study on formulation and stability characterization of monodisperse oil-in-water emulsions encapsulating quercetin

Nauman Khalid [a,b,1], Isao Kobayashi [a,*], Marcos A. Neves [a,c], Kunihiko Uemura [a], Mitsutoshi Nakajima [a,c], Hiroshi Nabetani [a,b]

[a] Food Research Institute, NARO, 2-1-12 Kannondai, Tsukuba, Ibaraki 305-8642, Japan
[b] Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
[c] Graduate School of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki 305-8572, Japan

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ABSTRACT

The study used microchannel emulsification (MCE) to encapsulate quercetin in food grade oil-in-water (O/W) emulsions. A silicon microchannel plate (Model WMS 1-2) comprised of 10,300 discrete 10 × 104 μm microslots was connected to a circular microhole with an inner diameter of 10 μm. 1% (w/w) Tween 20 was used as optimized emulsifier in Milli-Q water, while 0.4 mg ml^{-1} quercetin in different oils served as a dispersed phase. The MCE was carried by injecting the dispersed phase at 2 ml h^{-1}. Successful emulsification was conducted below the critical dispersed phase flux, with a Sauter mean diameter of 29 μm and relative span factor below 0.25. The O/W emulsions remained stable in terms of droplet coalescence at 4 and 25 °C for 30 days. The encapsulation efficiency of quercetin in the O/W emulsions was 80% at 4 °C and 70% at 25 °C during the evaluated storage period.

1. Introduction

Quercetin (3,3′,4′,5,7-pentahydroxyflavanone) is categorized as a flavonol and belongs to the family of flavonoids (Ross & Kasum, 2002). By definition, quercetin is an aglycone (lacking an attached sugar) with a brilliant citron yellow colour that is entirely insoluble in water, sparingly soluble in oil medium, and readily soluble in a variety of polar solvents (Kelly, 2011). The solubility of quercetin in the aqueous phase can be greatly improved by attaching glycosyl groups at hydroxyl positions (Hollman et al., 1999). Flavonols are present in many vegetables, flowers, nuts and fruits (Häkkinen, Kärenlampi, Heinonen, Mykkänen, & Törrönen, 1999). They are also abundant in a variety of medicinal plants, such as *Ginko biloba*, *Solanum trilobatum,* and many others (Kelly, 2011). The estimated intake of flavonols ranges from 20 to 50 mg d^{-1}. Most of the dietary intake is as flavonol glycosides of kaempferol, myricetin and quercetin (Cao, Zhang, Chen, & Zhao, 2010).

Quercetin exhibits a wide range of biological activities, including anticancer, antioxidant, antitoxic, antithrombotic, anti-ageing, metal chelating and antimicrobial activities (Borska et al., 2010; Kelly, 2011). Similarly, it has an impact on obesity, sleep and mood disorders (Joshi, Naidu, Singh, & Kulkarni, 2005; Kelly, 2011). Recently, quercetin has been used in many sport supplements in order to reduce post-exercise immune system perturbations (Davis, Carlstedt, Chen, Carmichael, & Murphy, 2010). The bioavailability and absorption of quercetin depend upon the nature of the attached sugar, solubility modifications, and the types of emulsifiers used in different systems (Scholz & Williamson, 2007). Despite its significant biological activities, quercetin has very poor oral bioavailability. The main disadvantages of using quercetin in therapeutics and functional foods are its poor solubility in aqueous and oil media, very low bioavailability, poor permeability and crystallization at ambient temperatures (Borghetti, Lula, Sinisterra, & Bassani, 2009; Pouton, 2006). To overcome these disadvantages, it is essential to develop an efficient delivery system for quercetin that improves its stability and release at the appropriate target site.

Different colloidal systems are there to encapsulate vital lipophilic compounds, including emulsions, solid lipid micro- and

* Corresponding author.
E-mail address: isaok@affrc.go.jp (I. Kobayashi).
[1] Present affiliation: Alliance for Research on North Africa, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki 305-8572, Japan; Algae Biomass and Energy System R&D Center, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki 305-8577, Japan; School of Agriculture, University of Management and Technology, Lahore, Pakistan.

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nano-particles, filled hydrogel particles and polymeric nano-particles (Flanagan & Singh, 2006; McClements & Rao, 2011). These colloidal delivery systems were formulated either with conventional emulsification tools or microfluidic devices (Vladisavljevic et al., 2013). In this study, we used microchannel emulsification (MCE) to encapsulate quercetin in different oil-in-water (O/W) emulsions. MCE is a promising technique for generating monodisperse emulsion droplets with a size variation of less than 5% (Kawakatsu, Kikuchi, & Nakajima, 1997). MCE devices consist of either parallel grooves and terraces or straight-through microholes (Kawakatsu et al., 1997; Kobayashi, Nakajima, Chun, Kikuchi, & Fujita, 2002). The distinguishing features of MCE involve the absence of external shear forces during droplet generation and the droplet size being mainly determined by the MC geometry and composition of dispersed and continuous phase (Vladisavljevic, Kobayashi, & Nakajima, 2012). The droplet generation in MCE takes place due to spontaneous transformation of a dispersed phase passing through the MCs, as a result of the interfacial tension dominant on micron scales (Sugiura, Nakajima, Iwamoto, & Seki, 2001). MCE has been successfully applied to the preparation of simple and multiple emulsions, microspheres and microcapsules (Vladisavljevic et al., 2013). Many hydrophilic and lipophilic compounds have been encapsulated in these systems, such as β-carotene (Neves, Ribeiro, Kobayashi, & Nakajima, 2008), oleuropein (Souilem et al., 2014), γ-oryzanol (Neves, Ribeiro, Fujiu, Kobayashi, & Nakajima, 2008), L-ascorbic acid (Khalid et al., 2014b, 2015a, 2015b), ascorbic acid derivatives (Khalid et al., 2014a) and vitamin D (Khalid et al., 2015a, 2015b).

The aim of this study was to design food grade O/W emulsions encapsulating quercetin using straight-through MCE. The present study investigated the effects of emulsifier type on the droplet generation characteristics and stability of emulsions encapsulating quercetin. Moreover, the effects of different dispersed phase composition on quercetin encapsulation were examined, together with the physical and chemical stability of the formulated emulsions. The results of this study improve the understanding of significant factors that influence the encapsulation, stabilization and utilization of crystalline bioactive compounds in food, cosmetics and pharmaceuticals.

2. Materials and methods

2.1. Chemicals

3,3′,4′,5,7-pentahydroxyflavanone (quercetin) was procured from Nacalai Tesque, Inc. (Kyoto, Japan). Dimethyl sulfoxide, polyoxyethylene (20) sorbitan monolaurate (Tween 20, Hydrophilic-Lipophilic Balance (HLB) 16.7) and bovine serum albumin (BSA) were procured from Wako Pure Chemical Industries (Osaka, Japan). Sodium salt of colic acid with >97% (Na-cholate) was procured from Sigma Aldrich (St. Louis, MO, USA). The medium chain triacylglycerides (MCT, sunsoft MCT-7) composed of 25% capric acid and 75% caprylic acid and polyglyceryl-5-laurate (Sunsoft A-12E, HLB 15.6) were purchased from Taiyo Kagaku Co., Ltd. (Yokkaichi, Japan). Decaglycerol monolaurate (ML-750, HLB 14.8) was procured from Sakamoto Yakuhin Kogyo Co., Ltd (Osaka, Japan). Milli-Q water with a resistivity of 18 MΩ cm served as a continuous phase medium and to dissolve different emulsifiers.

2.2. Preparation of dispersed and continuous phases

A continuous phase was prepared by dissolving 1% (w/w) Tween 20, Na-cholate, ML-750, Sunsoft A-12E, or BSA in Milli-Q water at ambient temperature, stirring for 20 min and storing for 60 min before it was used in emulsification. A disperse phase was prepared by dissolving (0.1–0.6 mg ml^{-1}) quercetin in MCT at ambient temperature. Afterwards, the mixture was heated in a water bath at 90 °C with constant stirring for 40 min and ultrasonication (VS-100III, As One Co., Osaka, Japan) at 45 kHz for 10 min. The mixture was heated again for 40 min, followed by ultrasonication for 10 min. The completely dissolved quercetin solution was stored at ambient temperature for 40 min before the experiments were conducted.

2.3. Silicon microchannel array chip

The encapsulation experiment was conducted using a silicon 24 × 24 mm MC array chip (Model WMS 1-2; EP. Tech Co., Ltd., Hitachi, Japan) containing 10,313 MCs arranged within a 10 mm^2 central region. The MC array chip was 500 μm thick but was thinned to 100 μm in the central region (Fig. S1a). The four holes with a diameter of 1.5 mm at the corners of each chip permitted dispersed and continuous phase to flow beneath the plate. MC array chips were fabricated by repeated photolithography and deep-reactive-ion etching (DRIE) on a 5-in silicon wafer. Each MC had a 10 μm diameter circular microhole of 70 μm depth that was located on the outlet side, and a microslot (11 × 104 μm cross section and 21 μm depth, aspect ratio = 9) located on the inner side (Fig. S1b). Before the first usage, the MC array chip was surface-oxidized in a plasma reactor (PR500, Yamato Science Co. Ltd., Tokyo, Japan) to produce a hydrophilic silicon dioxide layer on the surface. After conducting each experiment, the MC chip was cleaned with neutral detergent and ethanol using the above-mentioned ultrasonic bath at a frequency of 100 kHz and was subsequently stored in Milli-Q water.

2.4. Emulsification procedure

Before each experiment was conducted, the MC array chip was degassed in a continuous phase using ultrasonication at 100 kHz for 20 min. Afterwards, the MC chip was mounted in an MC module compartment previously filled with the continuous phase. A schematic diagram of this experimental step is presented in Fig. 1a. The dispersed phase was injected through the MCs by a syringe pump (Model 11, Harvard Apparatus Inc., Holliston, USA) using a 10 ml glass syringe at a dispersed phase flux (J_d) ranging from 10 to 300 L m^{-2} h^{-1}, while the continuous phase was delivered from an elevated reservoir through the gap between the MC array chip and the cover slip. The droplet generation process was observed using a FASTCAM-1024 PCI high speed video system at 250 to 1000 fps (Photron Ltd., Tokyo, Japan) attached to a metallographic microscope (MS-511B, Seiwa Kougaku Sesakusho Ltd., Tokyo, Japan). Each MCE experiment was conducted for about 2 h. The droplet generation process during MCE is demonstrated in Fig. 1b.

2.5. Measurement and analysis

The particle size distribution in the resultant O/W emulsions encapsulating quercetin was measured using a light-scattering instrument (LS 13 320, Beckman Coulter, Fullerton, USA). The particle size analyzer utilizes polarization-intensity differential scattering technology to measure the particle size. The particle size analyzer is able to measure sizes ranging from 0.04 to 2000 μm. The particle size was expressed as Sauter mean diameter ($d_{3,2}$), while the width of the particle size distribution was expressed as a relative span factor (RSF), defined as:

$$\mathrm{RSF} = \frac{d_{90} - d_{10}}{d_{50}} \quad (1)$$

where d_{90} and d_{10} and d_{50} are the equivalent volume diameters at 90, 10 and 50% cumulative diameter, respectively.