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

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ABSTRACT

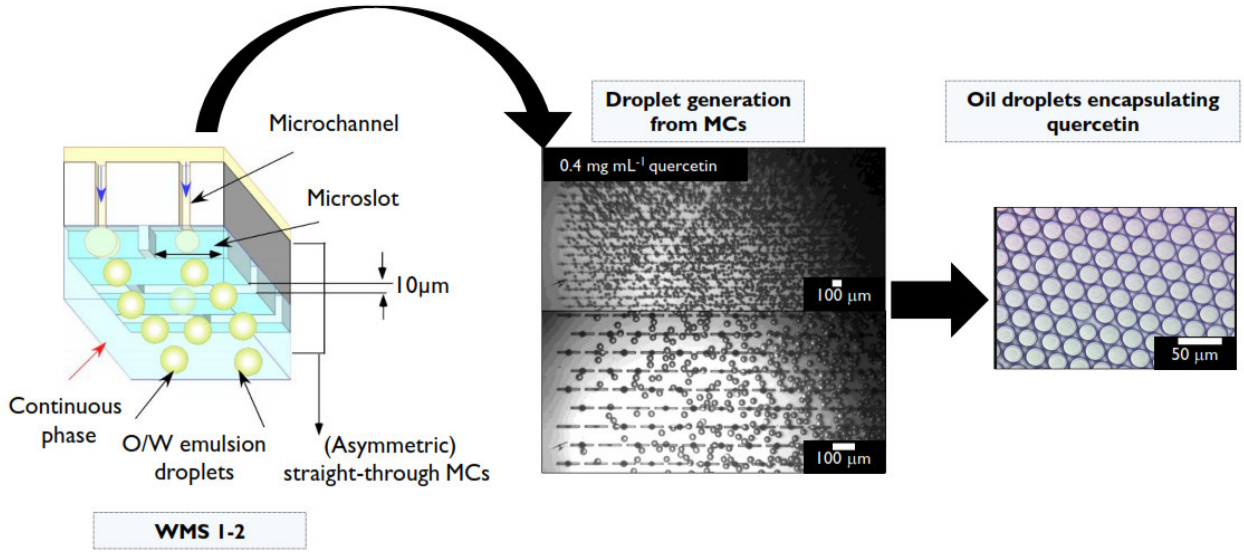
The study used microchannel emulsification (MCE) to encapsulate quercetin in food grade oil-in-water (O/W) emulsions. A silicon microchannel plate (Model WMS 1-2) comprised of 10,300 discrete 10 × 104 μm microslots was connected to a circular microhole with an inner diameter of 10 μm. 1% (w/w) Tween 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 ^{o}C for 30 days. The encapsulation efficiency of quercetin in the O/W emulsions was 80% at 4 ^{o}C and 70% at 25 ^{o}C during the evaluated storage period.

Keywords: Quercetin, microchannel emulsification, oil-in-water emulsions, emulsifiers, stability, droplet generation

Highlights

- Monodisperse O/W emulsions encapsulating quercetin were formulated.
- Uniformly sized droplets with $d_{3,2}$ of 28-29 μm were generated by MCE.
- Appropriate emulsion compositions and operating conditions for MCE were found.
- The collected O/W emulsions had high coalescence stability at 4 and 25 ^{o}C.
- Encapsulation efficiency of quercetin was about 80% after 30 d of storage at 4 ^{o}C.

Graphical abstract



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, Bijsman, van Gameren, Cnossen, de Vries, & Katan, 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 bioloba*, *Solanum trilobatum,* and many others (Kelly, 2011). The estimated intake of flavonols ranges from 20-50 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, Drag-Zalesinska, Wysocka, Sopel, Dumanska, Zabel, 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 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, Khalid, Neves, Kuroiwa, Nakajima, Uemura, 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, Kikuchi, & Nakajima, 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, Kobayashi, Neves, Sayadi, Ichikawa, & Nakajima, 2014), γ-oryzanol (Neves, Ribeiro, Fujiu, Kobayashi, &

Nakajima, 2008), L-ascorbic acid (Khalid, Kobayashi, Neves, Uemura, Nakajima, & Nabetani, 2014b, 2015a, 2015b), ascorbic acid derivatives (Khalid, Kobayashi, Neves, Uemura, Nakajima, & Nabetani, 2014a) and vitamin D (Khalid, Kobayashi, Wang, Neves, Uemura, Nakajima, 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 ^{o}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:

$$\text{RSF} = \frac{d_{90} - d_{10}}{d_{50}} \qquad (1)$$

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

2.6. Measurement of fluid properties

The densities of the different phases were measured using a density meter (DA-130 N, Kyoto Electronics Manufacturing Co., Ltd., Kyoto, Japan) at ambient temperature. The viscosities of the dispersed and continuous phases were measured with a sine wave vibro-viscometer (SV-10, A&D Co., Ltd., Tokyo, Japan) at ambient temperature. The interfacial tension between different phases were measured using a fully automatic interfacial tensiometer (PD-W, Kyowa Interface Sciences Co., Ltd., Niiza, Japan). The interfacial tensiometer works using a pendant drop method. Table S1 shows important physical properties of the dispersed and continuous phases.

2.7. Stability evaluation of O/W emulsions encapsulating quercetin

The physical stability in terms of consistency and coalescence was observed at 4 and 25 ± 1 °C with microscopic observations using an optical microscope (DM IRM, Leica Microsystems, Wetzlar, Germany), while the particle-size distribution and RSF during the storage period was measured using the above-mentioned light-scattering instrument.

The amount of quercetin present in the O/W emulsions and dispersed phases was quantified using a UV/VIS/NIR spectrophotometer (V-570, JASCO Co., Hachioji, Japan). Standard curves were prepared using stock solution of 1 mM quercetin and dimethyl sulfoxide (DMSO) as a

blank. The calibration curves were linear ($r^2 > 0.996$) over the quercetin concentration (1-10 μg ml^{-1}). The slope of the best-fit line was 0.066 ± 0.020 cm^{-1} (μg $ml^{-1})^{-1}$ and the intercept was 0.016 ± 0.00 cm^{-1}. The molar absorptivity ($\mathcal{E}$) for quercetin in this study was 21.86 mM^{-1} cm^{-1}.

The amount of quercetin encapsulated in the O/W emulsions was evaluated using absorbance measurements. One milliliter of sample from the middle of the bottle was dissolved in DMSO (ratio 1:10) then centrifuged at 3000 rpm for 15 min. DMSO was used to increase the solubility of quercetin in the aqueous phase. Aliquots of supernatant were filtered using a syringe filter with a mean pore size of 0.45 μm (Toyo Roshi Kaisha Ltd., Tokyo, Japan). The quercetin concentration was then determined by measuring the absorbance at 372 nm using a UV/VIS/NIR spectrophotometer. The encapsulation efficiency (EE_Q) of quercetin encapsulated in the O/W emulsions was calculated using the following equation:

$$EE_Q = \frac{C_{o,t}}{C_{o,i}} \times 100 \qquad (2)$$

where $C_{o,t}$ is the quercetin concentration that remained in the O/W emulsion at a specific time and $C_{o,i}$ is the initial quercetin concentration.

3. Results and discussion

3.1. Effect of different emulsifiers on emulsion formulation

Emulsifier molecules play a critical role in the stability of oil droplets in MCE. The charge on the MC array chip surface and its electrostatic interactions with the emulsifiers must be kept in mind during MCE. Uniformly sized oil droplets can be generated from the MCs when the chip surface has a non-attractive interaction with the emulsifier molecules. Moreover, the stability of the

droplets correlates with the type of emulsifier in the continuous phase and with whether it preferentially wets the chip surface during emulsification (Kobayashi, Nakajima, & Mukataka, 2003).

An MCT solution containing 0.4 mg ml^{-1} quercetin was used as the dispersed phase, while Milli-Q water containing 1% (w/w) emulsifier was used as the continuous phase. The MCE experiments were conducted by keeping J_d at 20 l m^{-2} h^{-1}, and the flow rate of the continuous phase (Q_c) was maintained between 250 and 500 ml h^{-1}. Stable generation of uniformly sized oil droplets was observed in all working MCs, regardless of the type of emulsifier used. There was neither the generation of irregularly sized droplets, nor a continuous growth of the dispersed phase, which could result in a broad size distribution and polydispersity (Fig. 2a). The formed droplets detached rapidly from the MC tip for all of the emulsifiers. Figure 2b depicts the $d_{3,2}$ and RSF of O/W emulsions stabilized by different emulsifiers. The $d_{3,2}$ of the O/W emulsions stabilized by nonionic emulsifiers (Tween 20, Sunsoft A-12E, and ML-750) ranged between 28.5 and 28.9 μm with an RSF of <0.21. Tween 20 was later used as optimized emulsifier in continuous phase due to better droplet generation and smooth detachment of droplets from MCs. As depicted in Fig. 2c, more stable droplet generation was observed with nonionic emulsifiers in the stable droplet-generation zone (J_d = 20-50 l m^{-2} h^{-1}) in comparison to anionic and protein-based emulsifiers (Na-cholate and BSA). A slighly greater $d_{3,2}$ value (29.4 μm) was observed for the O/W emulsions stabilized by Na-cholate, while those stabilized by BSA had a much greater $d_{3,2}$ (34.5 μm). The results with nonionic emulsifiers agreed well with previous emulsification studies reporting that a hydrophile-lipophile ratio exceeding 10 produced uniformly sized droplets in MCE (Kobayashi & Nakajima, 2002; Tong, Nakajima, Hiroshi, Nabetani, & Kikuchi, 2001). The effect of proteins as emulsifiers on MCE was previously

reported by Saito, Yin, Kobayashi, and Nakajima (2005). They described a slow increase in the average droplet diameter of BSA-stabilized emulsions with increasing J_d. Moreover, the generated emulsion droplets with BSA maintained monodispersity at high J_d. Our experiments with BSA were conducted at pH 7.1, indicating negatively charged BSA molecules. Their negative charge allows droplets to detach more smoothly from MCs (Saito, Yin, Kobayashi, & Nakajima, 2005).

3.2. Effect of quercetin concentration on emulsion formulation

To formulate an efficient delivery system, the most important task is to determine the loading capacity of the functional compound in the system. The physical properties of a functional compound have a major impact on bioavailability (Muller & Keck, 2004). The melting point of quercetin is relatively very high (>310 ^{o}C), and also it has low water solubility (< 1 g l^{-1}). These properties of quercetin limit its use in many food and pharmaceutical products (Borghetti, Lula, Sinisterra, & Bassani, 2009). MCT solutions containing quercetin with concentrations of 0.1 to 0.6 mg ml^{-1} remained stable for more than 30 days without showing any visible crystallization. To elevate the effect of quercetin concentration on O/W emulsions, the quercetin concentration in the dispersed phase ranged between 0.1 and 0.6 mg ml^{-1}. The continuous phase constituted 1% (w/w) Tween 20 in Milli-Q water. The dispersed phase was supplied into the MC module at J_d of 20 l m^{-2} h^{-1}. Effective MCE was conducted with different concentrations of quercetin. Figure 3a indicates the $d_{3,2}$ of O/W emulsions containing different concentrations of quercetin. The $d_{3,2}$ ranged from 28.6 to 29.2 μm with an RSF of <0.25. The narrow RSF indicates extreme monodispersity in the O/W emulsions encapsulating different concentrations of quercetin. There was smooth generation of emulsion droplets from the MCs regardless of the quercetin

concentrations applied at this stage (Fig. 3b), and these emulsions retained about 20-40 μg ml^{-1} of quercetin.

The quercetin solubility in the dispersed phase was slightly higher than previously reported by Pool, Mendoza, Xiao, and McClements (2013), which indicates nucleation of the quercetin in MCT beyond 0.1 mg ml^{-1}. However, such nucleation of quercetin was observed at 0.7 mg ml^{-1} in our study. Karadag, Yang, Ozcelik, and Huang (2013) reported two times higher solubility of quercetin in limonene oil in comparison to MCT when combined with Tween 80 and heated to 130 ^{o}C for 30 min.

3.3. Effect of dispersed phase flux and continuous phase flow velocity on emulsion formulation

MCE has a tendency to formulate monodisperse emulsions with no usage of energy. However, the low flux of the dispersed phase may be a restraining factor for MCE to be viable on an industrial scale.

Here the effect of various levels of J_d (10 to 300 l m^{-2} h^{-1}) and continuous phase velocity (2.8 to 22.8 mm s^{-1}) on droplet generation and the $d_{3,2}$ of the O/W emulsions formulated by MCE were evaluated. The flow state of dispersed and continuous phases at these velocities were completely laminar. The continuous phase flow velocity along the MC array chip surface ($\overline{V}_c$, mm s^{-1}) was determined as follows:

$$\overline{V}_c = \frac{Q_c}{A} \qquad (3)$$

where Q_c is the continous phase flow rate (mm^3 s^{-1}) and A is the flow area along the MC array chip surface (mm^2). Figure 4a shows the effect of the dispersed phase flux on the $d_{3,2}$ and RSF of the O/W emulsions encapsulating quercetin. The O/W emulsions were formulated using MCT solution containing 0.4 mg ml^{-1} quercetin as the dispersed phase, while Milli-Q water containing

1% (w/w) emulsifier was used as the continuous phase. The size-stable zone (the zone in which the droplet size exhibited small variation with increasing dispersed phase flow rate) had a J_d of 10-70 l m^{-2} h^{-1} (left of Fig. 4b). In this stable zone, $d_{3,2}$ was below 30 μm with an RSF of <0.35. At J_d above 80 l m^{-2} h^{-1}, the $d_{3,2}$ started to increase and entered a size-unstable zone. The emulsification at a J_d of above 100 l m^{-2} h^{-1} and a $\overline{V}_c$ of 22.8 mm s^{-1} resulted in the generation of nonuniform droplets with a $d_{3,2}$ of > 31.8 μm and an RSF of > 0.70 (right of Fig 4b). At higher J_d the $d_{3,2}$ depends on the oil velocity inside the MCs. The maximum J_d in the size-stable zone of quercetin-loaded emulsions was slightly higher than that in a previous study of γ-oryzanol-loaded O/W emulsions (Neves, Ribeiro, Fujiu, Kobayashi, & Nakajima, 2008). These authors reported a size-stable zone of J_d between 10 and 40l m^{-2} h^{-1} with a WMS 1-3 chip that has around 23,348 MCs. Moreover, in the current study a WMS 1-2 chip was used that has a total MC number of approximately half of that for WMS1-3. The maximum droplet generation number per active MC was assumed to be much higher in the present study. Moreover, the tendency of droplet size to increase in the size-unstable zone also agreed well with previous literature (Goran T. Vladisavljevic, Kobayashi, & Nakajima, 2011). Figure 5c depicts the effect of $\overline{V}_c$ on the $d_{3,2}$ and RSF of O/W emulsions encapsulating quercetin. The monodisperse O/W emulsions formulated at the $\overline{V}_c$ applied here showed no prominent difference in their $d_{3,2}$ and RSF. These trends are reasonable, since the resultant droplets with a diameter of <100μm are normally insensitive to $\overline{V}_c$ in MCE (Kobayashi, Hori, Uemura, & Nakajima, 2010).

3.4. Physical and chemical stability of O/W emulsions encapsulating quercetin

Monodisperse O/W emulsions encapsulating 0.4 mg ml^{-1} quercetin in the dispersed phase and stabilized with 1% (w/w) Tween 20 were stored at 4 and 25 ± 1 °C for a period of 30 d. A 0.4 mg

ml^{-1} solution of quercetin was used as the optimized concentration, since no crystalization was observed at this concentration even after 30 d of storage at 4 and 25 ± 1 °C. The emulsions were formulated by keeping J_d at 20 l m^{-2} h^{-1} and Q_c at 250 ml h^{-1}. Figure 5 illustrates the variation in $d_{3,2}$ and the RSF of the O/W emulsions encapsulating quercetin during storage at at 4 ± 1 °C or 25 ± 1 °C. Optical micrographs of the oil droplets after 30 d of storage at 4 and 25 °C are presented in Fig. 5b. The monodispersity can be clearly observed in these micrographs. There was hardly any change in their $d_{3,2}$ regardless of the storage temperature. The formulated O/W emulsions maintained their droplet size monodispersity at an RSF < 0.28 after 30 days of storage.

EE_Q was investigated for O/W emulsions encapsulating quercetin and stabilized with 1% (w/w) Tween 20. These emulsions were formulated by keeping J_d at 20 l m^{-2} h^{-1} and Q_c at 500 ml h^{-1}. The variation of EE_Q in the collected O/W emulsions during storage is depicted in Fig. 6. The freshly formulated O/W emulsions contained about 31.50 µg ml^{-1} of quercetin. There were gradual decreases in EE_Q regardless of the storage temperatures applied. The EE_Q values were 79.7% and 70.2% after 30 d of storage at 4 °C and 25 °C, respectively. These high EE_Q values can be attributed to the narrow size droplet distribution. Moreover, in MCE the droplet generation is based upon spontaneous tansformation of interfaces, that is further driven by differences in interfacial tension rather than the high-energy shear forces applied in mechanical homogenization. The energy efficiency (E_e) in MCE using the WMS 1-2 can be estimated by the following equation:

$$\Delta E_{exp} = \frac{\Delta P_d}{\rho_d} \qquad (4)$$

where ΔE_{exp} is the actual energy input for droplet generation in the straight-through MCE, ΔP_d is the applied dispersed phase pressure (4.6 kPa) during MCE for one hour, and ρ_d is the density of

the dispersed phase used here (Table S1).The calculated ΔE_{exp} for dispersed phase during emulsification was 4.9 J kg^{-1}. Equation 4 can also be used for calculating ΔE_{exp} for the continuous phase and ΔE_{exp} was 2.9 J kg^{-1}. The total ΔE_{exp} in MCE is a sum of the energy required for the dispersed phase flow and the energy required for the continuous phase flow and the calculated ΔE_{exp} was 7.8 J kg^{-1}. The ΔE_{exp} was comparable to the oblong-type straight-through MCE (5.0-14.2 J kg^{-1}), previously reported by Kobayashi, Takano, Maeda, Wada, Uemura, and Nakajima (2008). ΔE_{exp} must exceed the theoretical minimum energy (ΔE_{Thr}). ΔE_{Thr} is calculated using the following equation:

$$\Delta E_{Thr} = \Delta A \gamma_{ow} = \frac{6\gamma_{ow}}{\rho_d d_{3,2}} \quad (5)$$

where ΔA is the increase in interfacial area, γ_{ow} is the interfacial tension between the two phases, ρ_d is the dispersed phase density, and $d_{3,2}$ is the Sauter mean diameter of O/W emulsions. The ΔE_{Thr} at ambient temperature corresponds to 1.35 J kg^{-1}. E_e can be calculated from the following equation:

$$E_e = \frac{\Delta E_{Thr}}{\Delta E_{exp}} \times 100. \quad (6)$$

The calculated E_e was 17.3%, comparatively less than the E_e of the oblong-type straight-through MCE (Kobayashi, Takano, Maeda, Wada, Uemura, & Nakajima, 2008). The E_e of oblong-type MCE setups was 47-60% (Sugiura, Nakajima, Iwamoto, & Seki, 2001) and that for a grooved-type MCE was 65% (Kobayashi, Takano, Maeda, Wada, Uemura, & Nakajima, 2008; Sugiura, Nakajima, Iwamoto, & Seki, 2001). The E_e value obtained in this study indicates a mild droplet generation process for MCE, which is considerably higher than that for mechanical emulsification devices. Such a high E_e may also contribute to high EE_Q values after 30 d of storage.

Quercetin was previously encapsulated in poly D, L-lactide (PLA) nanoparticles and was found to have an encapsulation efficiency of 96.7% (Kumari, Yadav, Pakade, Singh, & Yadav, 2010), although the authors did not study the storage stability of quercetin-loaded PLA nanoparticles. Tan, Liu, Guo, and Zhai (2011) investigated lecithin-chitosan nanoparticles as a topical delivery system for quercetin. The quercetin-loaded nanoparticles initially showed higher permeation ability with an entrapment efficiency of 48.5%. Pool, Mendoza, Xiao, and McClements (2013) encapsulated 0.1 mg ml^{-1} quercetin in O/W emulsions and observed 95% retention at different storage temperatures; however, they noticed a considerable decrease in the level of quercetin in O/W emulsions containing 0.5 mg ml^{-1} quercetin after one month of storage. The above-mentioned encapsulation techniques involving mechanical emulsification promote the coalescence of polydisperse droplets during the storage period, which enhances the reduction in the encapsulation efficiency of the quercetin. In the current study, droplet monodispersity was very well maintained during the evaluated storage period. The unique microstructure design of MCE enables the production of monodisperse emulsion droplets. MCE produces these monodisperse droplets solely by spontanenous transformation of the oil-water interfaces. These advantages of MCE are expected to provide a breakthrough in the encapsulation of quercetin in the near future.

4. Conclusions

The present study demonstrated the successful formulation of food-grade monodisperse O/W emulsions encapsulating quercetin through straight-through MCE. The results for the effect of different emulsifiers indicate that Tween 20 was the best selection as the emulsifier for this study. Successful droplet generation and monodispersity were achieved at a maximum of 0.4 mg

ml^{-1} quercetin in an MCT oil as the dispersed phase. The selected operating parameters include J_d of 20-40 l m^{-2} h^{-1} and $\overline{V}_c$ of 5.0-20.0 mm s^{-1}. The formulated O/W emulsions encapsulating quercetin had an EE_Q exceeding 70% after 30 d of storage at refrigerated and ambient temperatures. The improvement in physical and chemical stability could be attributed to their high monodispersity as well as extremely mild droplet generation by MCE. The formulation of new high energy drinks containing natural ingredients like quercetin is an innovation that could arise due to the current study. This research is also applicable for designing new functional products. However, designing these products are beyond the scope of present study. The results for this study specify that MCE is a promising technique to encapsulate a variety of bioactive compunds in a more controlled manner.

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Figure and Table captions

Fig. 1. Schematic presentation of MCE used in the study. (a) Experimental setup used for quercetin encapsulation. (b) Oil droplet generation process in MCE.

Fig. 2. Effect of different emnulsifiers on the generation of O/W emulsions encapsulating quercetin in straight-throught MCE (a) and on their $d_{3,2}$ and RSF (b). ▭ denotes the $d_{3,2}$ of O/W emulsions and ● denotes the RSF.

Fig. 3. (a) Effect of quercetin concentration on the $d_{3,2}$ and RSF of O/W emulsions. Solid keys denote $d_{3,2}$ and open keys denote RSF. (b) Optical micrographs of oil droplet generation at different concentrations of quercetin (0.1 mg ml^{-1} in (i) and 0.6 mg ml^{-1} in (ii)).

Fig. 4. (a) Effect of disperse phase flux on $d_{3,2}$ and RSF of O/W emulsions encapsulating quercetin. (b) Optical micrographs of oil droplet generation at disperse phase fluxes of 30 to 300 l m^{-2} h^{-1}. Continuous phase velocity was between 14 and 23 mm s^{-1}. (c) Effect of continuous phase flow velocity on $d_{3,2}$ and RSF of O/W emulsions encapsulating quercetin at a disperse phase flux of 20 l m^{-2} h^{-1}. Solid keys in (a) and (c) denote $d_{3,2}$ and open keys in (a) and (c) denote RSF.

Fig. 5. (a) Storage stability of O/W emulsion droplets encapsulating querectin. The data is presented in terms of $d_{3,2}$ and RSF. (-●-) denotes storage stability at 4°C and (-▲-) denotes storage stability at 25°C. The same open keys denote the RSF of O/W emulsions encapsulating quercetin. (b) Optical micrographs of O/W emulsions encapsulating quercetin after 30 days of storage at 4 and 25°C.

Fig. 6. Encapsulation efficiency (EE_Q) and retention profile (R_Q) of O/W emulsions encapsulating quercetin stored at 4 and 25°C. (-●-) denotes R_Q at 4°C and (-▼-) denotes R_Q at 25°C. (-⊖-) represents EE_Q at 4°C and (-▽-) represents EE_Q at 25°C.

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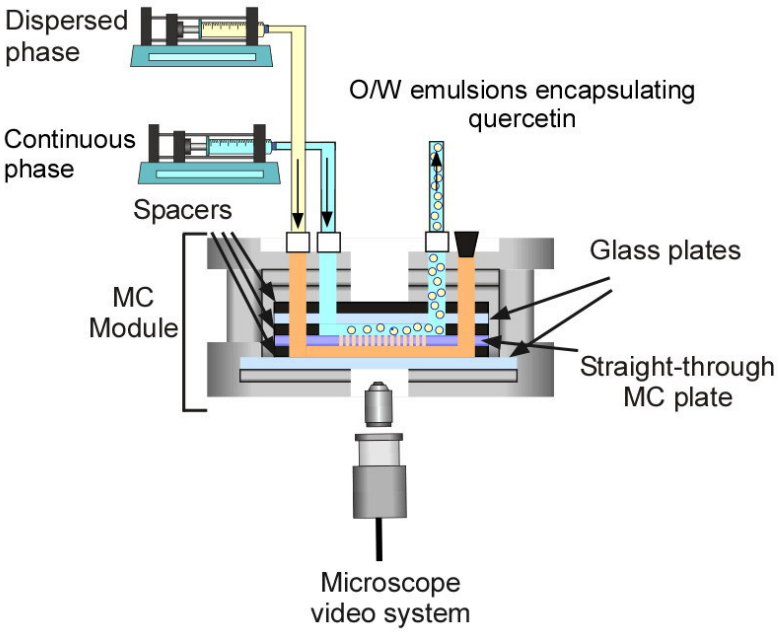
(a)



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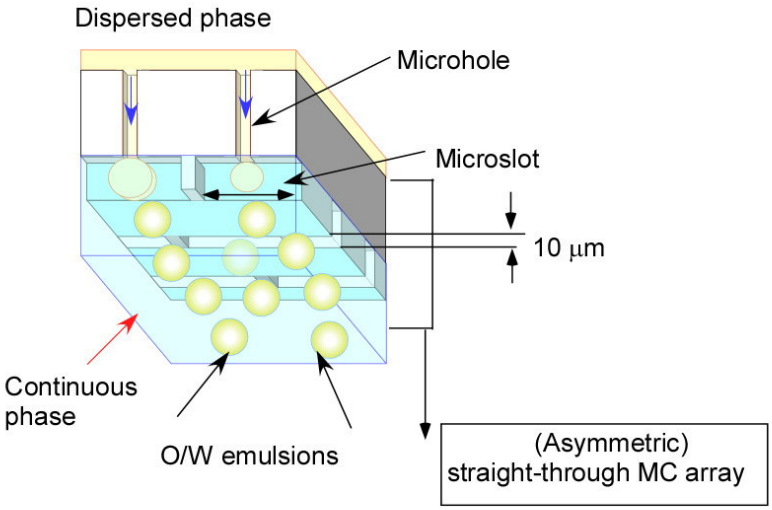
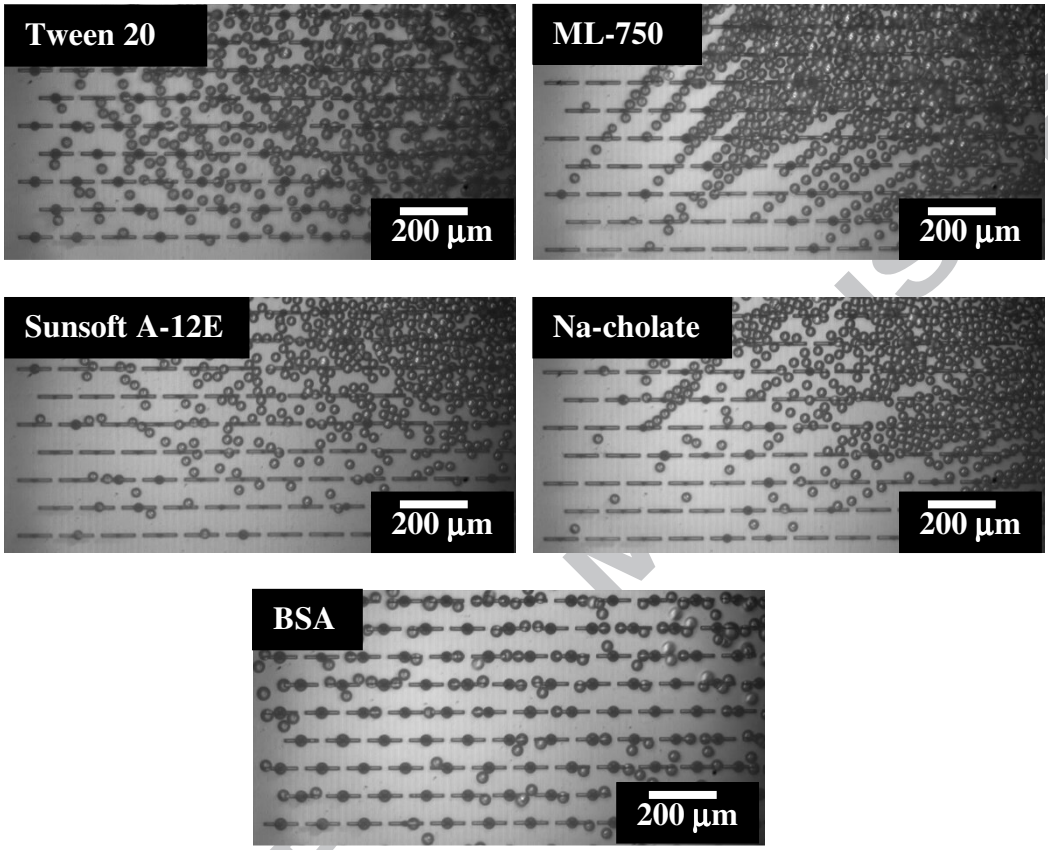


Figure 1

(a)



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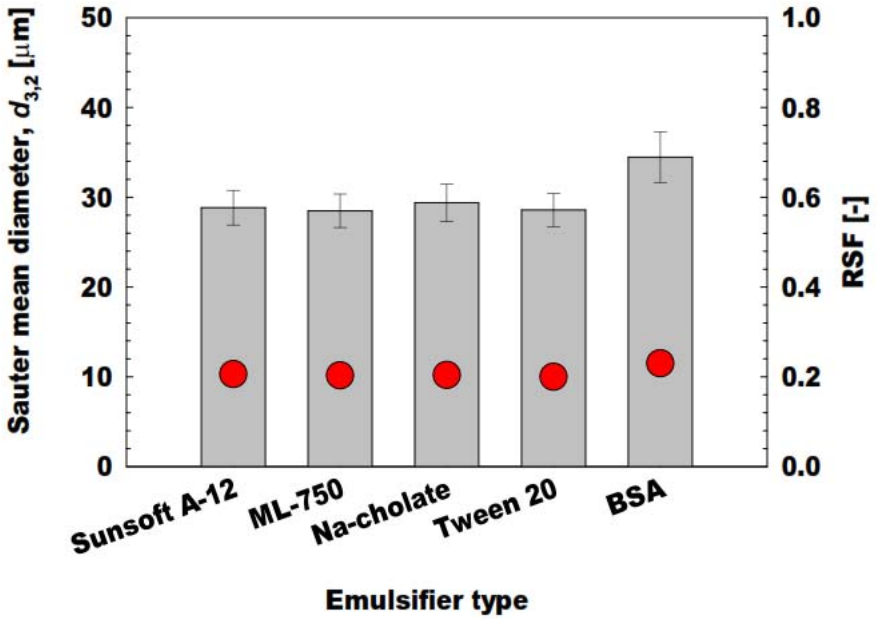
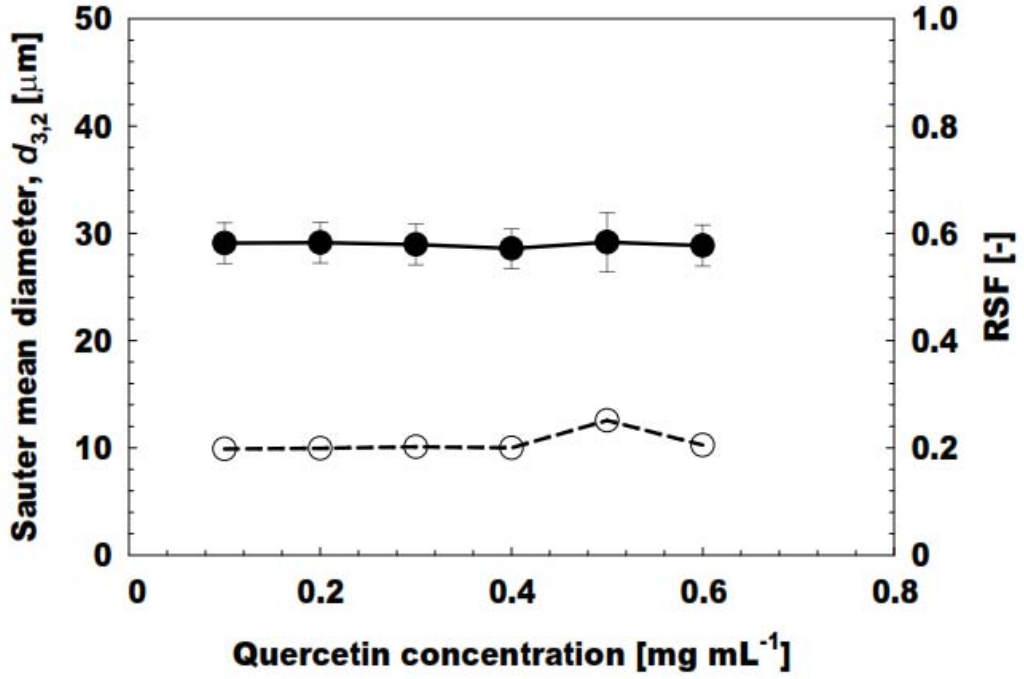

Sauter mean diameter, $d_{3,2}$ [μm]
RSF [-]
Sunsoft A-12
ML-750
Na-cholate
Tween 20
BSA
Emulsifier type

Figure 2

(a)


Sauter mean diameter, $d_{3,2}$ [μm]
RSF [-]
Quercetin concentration [mg mL^{-1}]

(b)

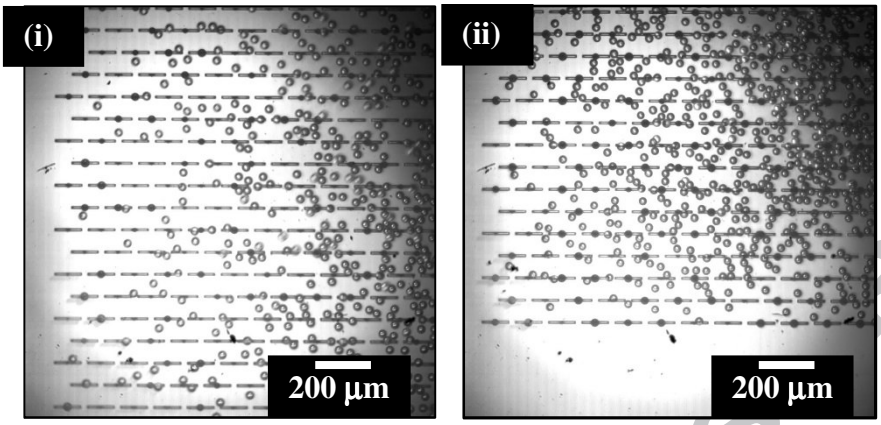
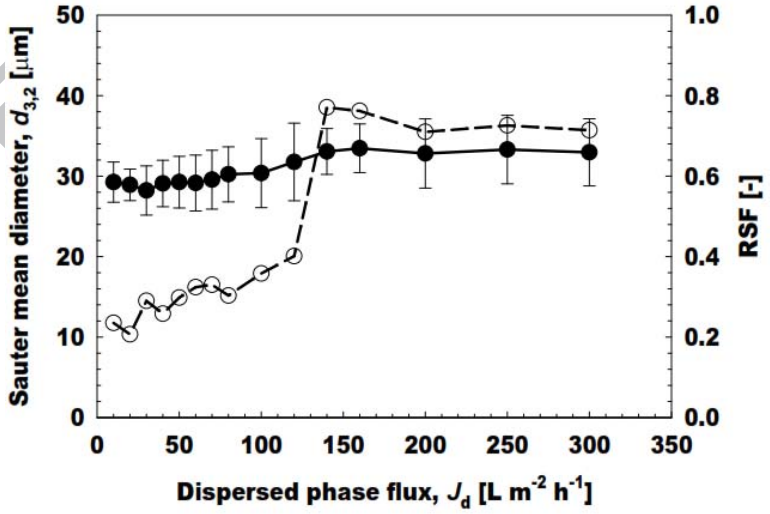
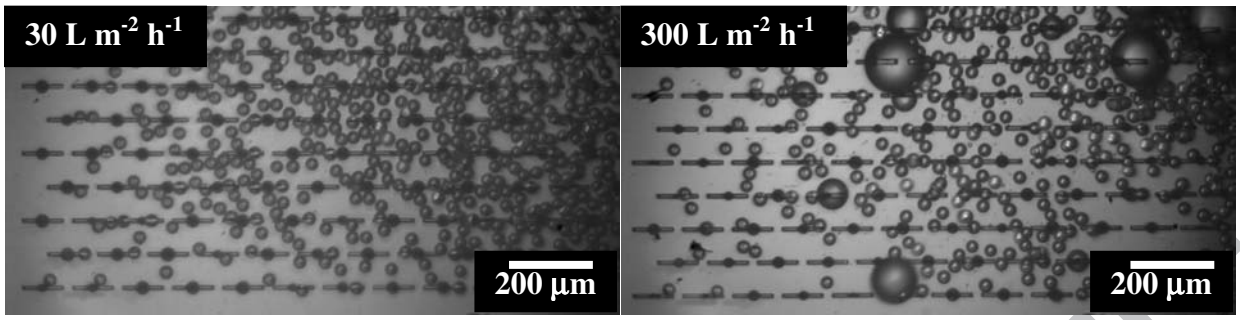


Figure 3

(a)



(b)


30 L m-2 h-1
200 µm
300 L m-2 h-1
200 µm

(c)

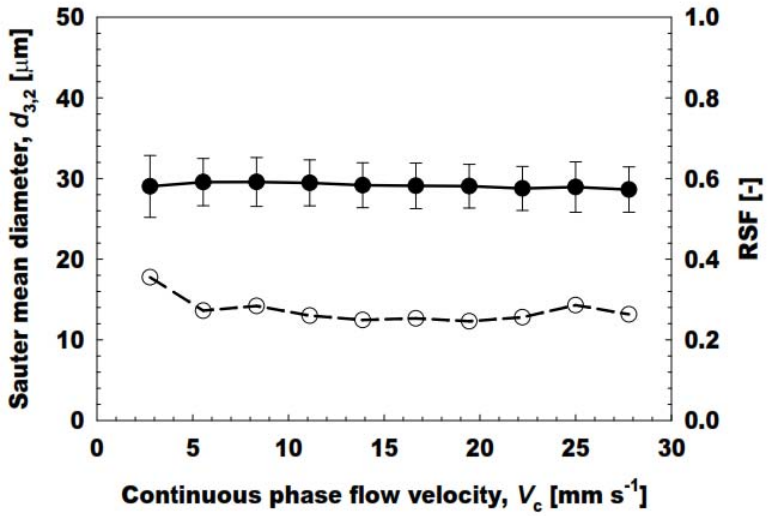
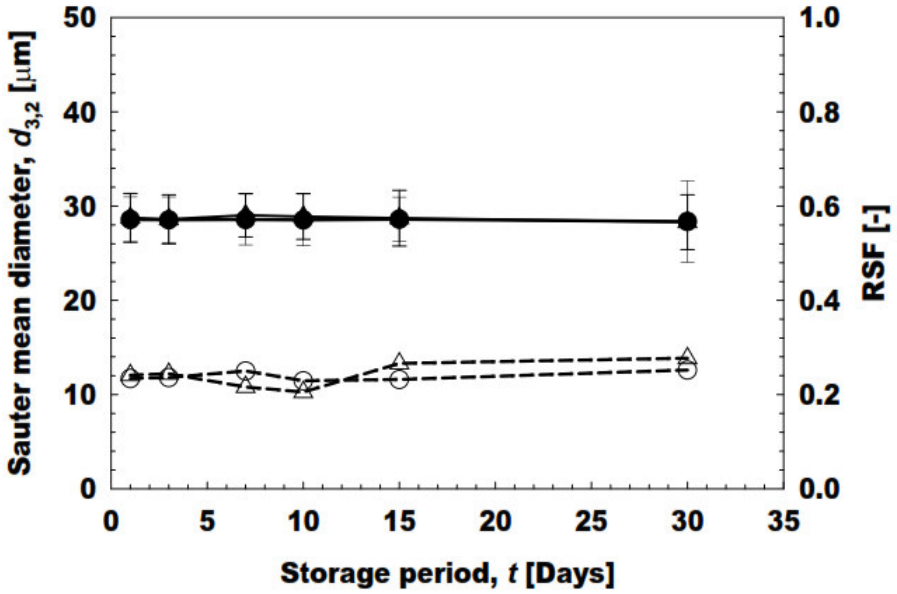

Sauter mean diameter, $d_{3,2}$ [µm]
RSF [-]
Continuous phase flow velocity, V_c [mm s^{-1}]
0
10
20
30
40
50
0.0
0.2
0.4
0.6
0.8
1.0
0
5
10
15
20
25
30

Figure 4

(a)


Sauter mean diameter, $d_{3,2}$ [μm]
RSF [-]
Storage period, t [Days]
0
10
20
30
40
50
0.0
0.2
0.4
0.6
0.8
1.0
0
5
10
15
20
25
30
35

(b)

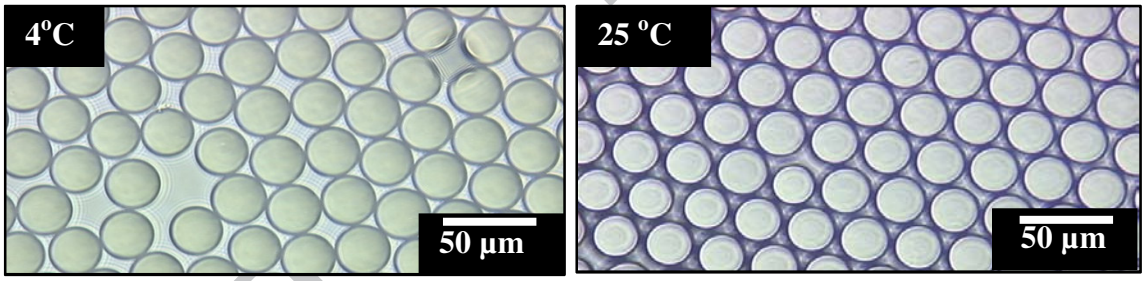

4°C
50 μm
25 °C
50 μm

Figure 5

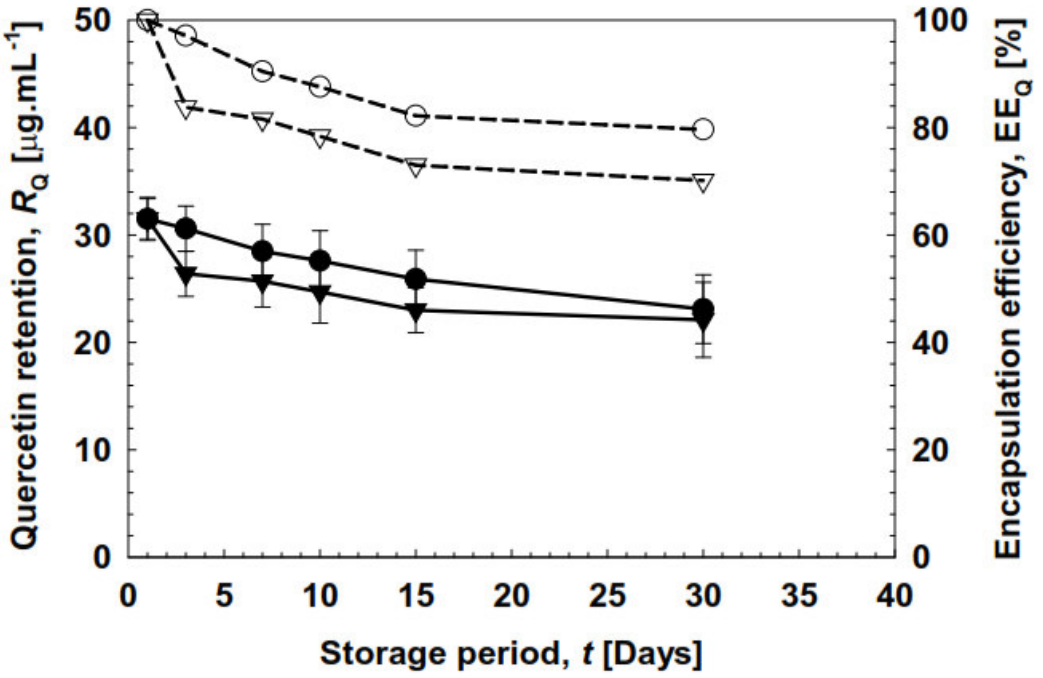


Figure 6

Highlights

- Monodisperse O/W emulsions encapsulating quercetin were formulated.
- Uniformly sized droplets with $d_{3,2}$ of 28-29 μm were generated by MCE.
- Appropriate emulsion compositions and operating conditions for MCE were found.
- The collected O/W emulsions had high coalescence stability at 4 and 25 ^{o}C.
- Encapsulation efficiency of quercetin was about 80% after 30 d of storage at 4 ^{o}C.