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Co-delivery of hydrophobic curcumin and hydrophilic catechin by a water-in-oil-in-water double emulsion

N.P. Aditya [a], Sheetal Aditya [a], Hanjoo Yang [a], Hye Won Kim [a], Sung Ook Park [b], Sanghoon Ko [a,*]

[a] Department of Food Science and Technology, Sejong University, 98 Gunja-dong, Gwangjin-gu, Seoul 143-747, Republic of Korea
[b] School of Management, Kyung Hee University, 1 Hoegi-dong, Dongdaemoon-gu, Seoul 130-701, Republic of Korea

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ABSTRACT

Curcumin and catechin are naturally occurring phytochemicals with extreme sensitivity to oxidation and low bioavailability. We fabricated a water-in-oil-in-water (W/O/W) double emulsion encapsulating hydrophilic catechin and hydrophobic curcumin simultaneously. The co-loaded emulsion was fabricated using a two-step emulsification method, and its physicochemical properties were characterised. Volume-weighted mean size (d_{43}) of emulsion droplets was ≈3.88 μm for blank emulsions, whereas it decreased to ≈2.8–3.0 μm for curcumin and/or catechin-loaded emulsions, which was attributed to their capacity to act as emulsifiers. High entrapment efficiency was observed for curcumin and/or catechin-loaded emulsions (88–97%). Encapsulation of catechin and curcumin within an emulsion increased their stability significantly in simulated gastrointestinal fluid, which resulted in a four-fold augmentation in their bioaccessibility compared to that of freely suspended curcumin and catechin solutions. Co-loading of curcumin and catechin did not have adverse effects on either compound's stability or bioaccessibility.

1. Introduction

In the last few decades, increasing evidence has emerged linking "nutraceuticals" with health promotion and disease prevention. Interestingly, recent studies have shown the possibility of reducing cancer deaths by 30% by the adoption of healthy lifestyles and dietary modifications (Liu, 2013). Although nutraceuticals can control diseases, such as diabetes mellitus and obesity, they have been used primarily as alternative or complimentary medicines rather than primary treatments (Braithwaite et al., 2014). The main reason for such secondary use is their low solubility, stability and cell permeability in the gastrointestinal tract (GIT) (Braithwaite et al., 2014). These properties result in low and inconsistent bioaccessibility (quantity or fraction of the ingested bioactive or molecules that is available for absorption), which in turn compromise their biological activity (Aditya et al., 2014).

Curcumin is a hydrophobic yellow pigment isolated from *Curcuma longa Linn*. It is used extensively in food, cosmetics and medicines. Catechin is natural hydrophilic antioxidant molecule used extensively in foods and medicines (Aditya et al., 2013; Rashidinejad, Birch, Sun-Waterhouse, & Everett, 2014). Recent studies have shown synergism between curcumin and catechin in disease prevention and health promotion. In the recent study, curcumin and catechin combination is shown to inhibit cell proliferation through induction of apoptosis (Manikandan et al., 2012). In another study, curcumin and catechin combination successfully reduced colorectal aberrant crypt foci (ACF) in colorectal cancer induced male Wister rats (Xu et al., 2010).

Curcumin and catechin suffer from low stability and pharmacokinetic mismatch that compromises their potency (Aditya et al., 2013; Rashidinejad et al., 2014). In the case of curcumin, low bioavailability is a result of its low solubility in water, degradation in alkaline pH conditions, inadequate absorption, and rapid removal from the body (Anand, Kunnumakkara, Newman, & Aggarwal, 2007). In the case of catechin, low bioavailability is a result of its limited membrane permeability, degradation in digestive conditions (pH 6–8), and transporter-mediated intestinal secretion (Cai, Anavy, & Chow, 2002).

In recent years, several attempts have been made to increase the therapeutic potential of these nutraceuticals by using bioavailability enhancers, combining the two nutraceuticals to elicit synergistic actions, and developing delivery systems (e.g., liposomes, emulsions, lipid nanocarriers, and polymeric carriers). For example, nano sized protein (β-lactoglobulin) and EGCG complexes were fabricated to delay the oxidation of EGCG molecules in aqueous media (Shpigelman, Israeli, & Livney, 2010). In another couple

* Corresponding author. Tel.: +82 (2) 3408 3260; fax: +82 (2) 3408 4319.
E-mail addresses: adi_nayak2003@yahoo.co.in (N.P. Aditya), sheetal.7248@gmail.com (S. Aditya), hjyang@sju.ac.kr (H. Yang), hyewonwawa@naver.com (H.W. Kim), sopark@khu.ac.kr (S.O. Park), sanghoonko@sejong.ac.kr (S. Ko).

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of studies, catechin has been entrapped using elastic liposomes and chitosan–tripolyphosphate nanoparticles (CS NPs) which have shown greater stability of catechin in gastro intestinal fluid (GIT) and enhanced bioavailability (Dube, Nicolazzo, & Larson, 2011; Huang et al., 2011; Ting, Jiang, Ho, & Huang, 2014). Efforts have focused on fabricating delivery systems to deliver curcumin or catechin individually. The main hurdle is the difference in their solubility. Curcumin is highly hydrophobic and soluble in lipids, whereas catechin is hydrophilic and insoluble in lipids. Thus, the carrier that suits catechin delivery will not be suitable for the delivery of curcumin. Thus, development of a co-loaded delivery system that can encapsulate curcumin and catechin and protect them from degradation until they reach the site of action would be novel and beneficial. Such a delivery system would increase their bioaccessibility and biological activity. Recent studies from our research team and other scholars have shown the benefits of delivering a combination of nutraceuticals by using co-loaded nanocarriers (Aditya et al., 2013; Tavano, Muzzalupo, Picci, & de Cindio, 2014).

A water-in-oil-in-water (W/O/W) double emulsion was fabricated to explore its unique nature of accommodating hydrophilic and hydrophobic molecules. The W/O/W double emulsion contains a W_1 phase comprising water and other stabilisers that are dispersed as small water particles in the lipid phase. This constitutes the oil phase of the system and it separates the internal water phase from the external water phase (W_2), which comprises a surfactant and other stabilizers (Qi, Wang, & Zhu, 2011). In the present study, the internal water phase (W_1) was used to entrap the hydrophilic catechin, and the oil phase was used to entrap the hydrophobic curcumin. Studies have shown the increased protection, controlled release, and enhanced bioavailability of molecules entrapped in W_1, and the presence of lipids is known to increase the bioaccessibility of curcumin by forming micelles after intestinal digestion and protect curcumin from the adverse conditions of the GIT (Aditya et al., 2013; Koga, Takarada, & Takada, 2010; Qi et al., 2011). To our knowledge, this is the first study in which a dual nutraceutical-loaded W/O/W emulsion was fabricated to deliver hydrophilic and hydrophobic molecules simultaneously.

The interrelated objectives of the present study were to fabricate a curcumin and catechin co-loaded W/O/W emulsion to (a) achieve controlled release, (b) increase the stability of curcumin and catechin in the GIT, and (c) enhance the bioaccessibility of curcumin and catechin. The fabricated double emulsions were characterised for their physicochemical properties: size, zeta potential, encapsulation efficiency, stability, bioaccessibility, and *in vitro* drug release.

2. Materials and methods

2.1. Materials

Curcumin (purity, >95%) and catechin (>98%) were purchased from Sigma–Aldrich (Saint Louis, MO, USA). Olive oil was obtained from Dae Jung Chemicals (Seoul, Korea). Porcine bile extract, gelatin, and NaCl were purchased from Sigma–Aldrich. All other chemicals were of analytical grade.

2.2. Quantification of curcumin and catechin by high-performance liquid chromatography (HPLC)

A HPLC method for simultaneous quantification of curcumin and catechin was developed using a 1200 HPLC system (Agilent Technologies, Santa Clara, CA, USA) controlled by Chem Station software (Hewlett Packard, Wilmington, DE, USA) equipped with an auto sampler and analytical Discovery HSC18 5-μm column (25 cm × 4.6 mm; (Supelco, Bellefonte, PA, USA). The mobile phase for detection of curcumin and catechin was 5% acetic acid and acetonitrile (25:75), which was eluted isocratically at 1.0 ml/min. Curcumin and catechin were detected at 424 nm and 280 nm, respectively. Curcumin and catechin were detected at a column temperature of 25 °C. Linearity was found in the range 0.1–8 μg/ml ($r^2 = 0.999$) for curcumin and 1–10 μg/ml ($r^2 = 0.998$) for catechin.

2.3. Preparation of a W/O/W double emulsion

A two-step emulsification method was employed to prepare a W/O/W double emulsion as described earlier with modification (Qi et al., 2011). To fabricate a W/O emulsion, olive oil (oil phase) was heated to ≈60 °C after mixing with the hydrophobic surfactant polyglycerol polyricinoleate (hydrophilic–lipophilic balance [HLB], 0.6), curcumin (0.1% *w/v*) and stirred for 15 min using a magnetic stirrer. The internal aqueous phase (W_1) comprising catechin (750 μg/ml), gelatin (3%), NaCl (2%), and ascorbic acid (0.2%) was heated to ≈60 °C. The W/O emulsion was fabricated by drop-wise dispersion of the water phase into the oil phase. The ratio of the water phase to the oil phase was 25:75. This primary W/O emulsion was stirred using a magnetic stirrer at 1500 rpm for 2 min and sonicated using a Probe Sonicator (SON-1VCX130, Sonics and Materials, Newton, CT, USA) at a frequency of 20 kHz and amplitude of 40% (work time, 3 s; rest time, 3 s) for 4 min. The fabricated W/O emulsion was stored for 30 min at 4 °C to allow sol–gel transition of gelatin. A blank W/O emulsion was fabricated without adding catechin in the W_1 phase or curcumin in the oil phase. The primary W/O emulsion was brought to room temperature (25 ± 2 °C) and added to the secondary water phase (W_2), which comprised the hydrophilic surfactant Tween 80 (1%; HLB, 15), ascorbic acid (0.2%) and NaCl (2%) under magnetic stirring at 1500 rpm. After 15 min of stirring, it was processed further using a probe sonicator at a frequency of 20 kHz and amplitude of 30% (work time, 3 s; rest time, 3 s) for 2 min. The ratio of the W/O phase to the W_2 phase was 25:75. The overall composition of the W/O/W emulsion for blank, curcumin delivery, catechin delivery, and co-delivery of curcumin and catechins shown in Table 1.

2.4. Optical microscopy

Freshly prepared W/O/W samples were diluted using deionised water (1:1000), placed on microscopic slides, covered with coverslips and observed under an Optical Microscope (Eclipse 80i; Nikon, Tokyo, Japan) at room temperature. Emulsions were observed to identify the basic characteristics of the double emulsion.

2.5. Measurement of particle size and zeta potential

Dynamic light scattering was used to evaluate the size of the W/O/W emulsion dispersed in deionised water using a Particle Size Analyzer (Mastersizer 2000S; Malvern Instruments, Malvern, UK) equipped with a He–Ne laser (623 nm). The device was programmed to count and calculate the size of particles with diameters of 0.02–2000 μm. W/O/W samples were diluted 1:1000 using deionised water and zeta potential measured using a commercial Zeta Potential and Particle Size Analyzer (Delsa Nano Particle Size; Beckman Coulter, Fullerton, CA, USA). Measurement of size and zeta potential were done in triplicate at least.

2.6. Encapsulation efficiency

The amount of curcumin and catechin entrapped as well as the amount loaded in their respective phases was determined using methods described previously with modification (O'Regan and

Table 1
Compositions of W/O primary emulsion and W/O/W double emulsion for blank, curcumin delivery, catechin delivery, and co-delivery of curcumin and catechin.

Emulsion types	Materials	Compositions (wt%) for different core deliveries			
		Blank	Curcumin	Catechin	Curcumin + catechin
W/O primary emulsion	Olive oil	75	75	75	75
	Catechin	0	0	0.075	0.075
	Curcumin	0	0.1	0	0.1
	Water	25	25	25	25
	Gelatin	3	3	3	3
	NaCl	2	2	2	2
	Ascorbic acid	0.2	0.2	0.2	0.2
W/O/W double emulsion	W/O emulsion	25	25	25	25
	Water	75	75	75	75
	Tween 80	1	1	1	1
	NaCl	2	2	2	2
	Ascorbic acid	0.2	0.2	0.2	0.2

Mulvihill, 2010; Qi et al., 2011). Briefly, 4 ml of curcumin, catechin, or a curcumin and catechin co-loaded W/O/W emulsion was placed in a Falcon™ tube and centrifuged at 16000×*g* for 4 min at 4 °C using a refrigerated centrifuge. The W_2 phase at the bottom of the Falcon tube (which contained unentraped curcumin and catechin) was collected using a syringe and passed through 0.22-μm syringe filter to collect only water and to exclude oil droplets. Collected samples were stored at −70 °C until analyses using the HPLC system described in Section 2.2.

Encapsulation efficiency was calculated using Eq. (1) for catechin and Eq. (2) for curcumin:

$$\text{EE } (\%) = \frac{N_{W_1} - N_{W_2}}{N_{W_1}} \times 100 \quad (1)$$

$$\text{EE } (\%) = \frac{N_{oil} - N_{W_1}}{N_{oil}} \times 100 \quad (2)$$

where N_{W_2} is the amount of catechin and/or curcumin seeping to the outer aqueous phase, N_{W_1} is the amount of catechin added to the inner aqueous phase and N_{oil} is the amount of curcumin added to the oil phase.

Loading efficiency (LE) was calculated using Eq. (3) for catechin and Eq. (4) for curcumin

$$\text{LE } (\%) \text{ of catechin} = \frac{\text{total catechin added in } W_1 - \text{catechin unentraped}}{\text{total water added in } W_1} \times 100 \quad (3)$$

$$\text{LE } (\%) \text{ of curcumin} = \frac{\text{total curcumin added in oil phase} - \text{curcumin unentraped}}{\text{Total oil added in oil phase}} \times 100 \quad (4)$$

Efficacy of the fabricated co-loaded emulsion for providing stability to curcumin and catechin in the stomach and intestine was tested using simulated intestinal fluid (SIF) and simulated gastric fluid (SGF)as described previously (Aditya et al., 2014; Xu et al., 2014), and compared with the stability of free curcumin and free catechin. Briefly, 5 ml of a curcumin and catechin co-loaded W/O/W emulsion or 5 ml of distiled water containing curcumin, catechin, ascorbic acid, NaCl, gelatin as that of same volume of double emulsion was mixed with 5 ml of SGF (2 g of NaCl and 7 ml of HCl dissolved in 1 l of water and pH adjusted to 2 ± 0.1 using 0.1 M HCl) or 5 ml SIF (bile extract solution 0.7 mg/ml and 1 ml of $CaCl_2$ solution (750 mM), pH adjusted to 7 ± 0.1 using 0.1 M NaOH) at 37 °C in a water bath shaking at 250 rpm for 2 h. At 30, 60 and 120 min, samples were withdrawn and diluted with ethanol (1:10) to solubilise curcumin or catechin, and centrifuged at 16,000×*g* for 20 min. The supernatant was passed through 0.22-μm syringe filters and analysed using HPLC.

2.7. In vitro release of nutraceuticals

Release of curcumin and/or catechin under the simulated intestinal condition without enzymes was carried out using Dialysis Membrane Bags (molecular weight cut-off, 6000–8000 Da; Spectra/Por, Rancho Dominguez, CA, USA) as described earlier (Aditya et al., 2014). Dialysis bags were soaked in water 3–4 h before use. Subsequently, bags were filled with 8 ml of W/O/W emulsion (curcumin, 1.32 mg; catechin, 0.37 mg) and placed in 30 ml releasing media comprising porcine bile extract (0.7 mg/ml), 1.7 ml $CaCl_2$ (750 mM) and 40% ethanol maintained at 37 °C using a water bath at 50 rpm/min. The sample (1 ml) was collected at 30, 60, 180 and 360 min and an identical amount of releasing media replaced to maintain the same volume of releasing media throughout the experiment. Collected samples were used to detect curcumin and/or catechin using HPLC after suitable dilution with ethanol.

2.8. In vitro bioaccessibility study

The bioaccessibility of curcumin and catechin was determined using an *in vitro* lipid digestion assay as described previously with slight modification (Aditya et al., 2013). To mimic gastric digestion, SGF was constituted using pepsin (0.32% w/v), NaCl (2 g) and HCl (7 ml), which were dissolved in 1 l of water and the pH adjusted to 2.0 ± 0.1 using 0.1 M HCl. Gastric digestion was initiated by adding 2.5 ml of emulsion (curcumin/catechin or co-loaded curcumin and catechin) or a native curcumin and catechin combination (with a concentration equal to the emulsion) suspended in phosphate-buffered saline to an equal volume of SGF. After thorough mixing, the pH was adjusted to 2.0 ± 0.1 using 0.1 M HCL and incubated at 37 ± 1 °C with constant shaking (250 rpm) for 2 h. After incubation for 2 h, samples were subjected to intestinal digestion. Here, digesta (5 ml) was mixed with an equal volume of SIF containing a final concentration of lipase (0.4 mg/ml), pancreatin (0.5 mg/ml) and other excipients; bile extract (0.7 mg/ml) and 1 ml of calcium chloride solution (250 mM), and the pH adjusted to 7.2 ± 0.1 using 1 M NaOH. This mixture was shaken at 100 rpm at 37 °C in a water bath. After incubation for 2 h, digesta was centrifuged at 3500 rpm for 30 min at 4 °C. The supernatant was collected and centrifuged at 14,000 rpm for 20 min, and 100 μl of the supernatant containing chyme (micellar or soluble fraction of curcumin and catechin) collected and diluted with ethanol at 1:10 to break the micelles and centrifuged again at 14,000 rpm for 20 min. The supernatant was used to quantify curcumin and catechin *via* HPLC

as described in Section 2.2. Bioaccessibility was calculated using the formula:

$$\text{Bioaccessibility } (\%) = \frac{\text{curcumin in the chyme fraction}}{\text{total curcumin added}} \times 100 \quad (5)$$

2.9. Statistical analyses

Values are shown as mean ± SD. Statistical analyses were done using the Student's *t*-test; $P < 0.05$ was considered significant.

3. Results and discussion

3.1. Morphology and droplet size of W/O/W double emulsions

Two-step emulsification methods were employed for the fabrication of double emulsions. To observe the physical appearance of fabricated W/O/W emulsions, light microscopy was used. Fabricated emulsions (blank, catechin, curcumin, and catechin + curcumin) clearly showed an internal aqueous phase (W_1) surrounded by oil droplets that were dispersed further in a secondary aqueous phase (W_2), thereby confirming the characteristics of double emulsions (Fig. 1A).

Immediately after preparation, the droplet-size distribution of oil globules in W/O/W emulsions was measured by dynamic light scattering (Table 2). Blank emulsion had a volume-weighted mean diameter (d_{43}) of 3.88 μm with unimodal size distribution. Encapsulation of curcumin and catechin resulted in a moderate decrease in d_{43} to 2.88 and 2.82 μm, respectively. Co-loading had no significant effect on emulsion size distribution ($d_{43} \approx 3.06$ μm). Similarly, the size below which 90% of the volume of droplets existed ($d(v)$ 90%) of the blank double emulsion was ≈12.1 μm whereas, after loading with curcumin and catechin, it was 6.6 and 8.0 μm, respectively. After loading curcumin and/or catechin, the proportion of size distribution < 1 μm increased (Fig. 1B). The observed

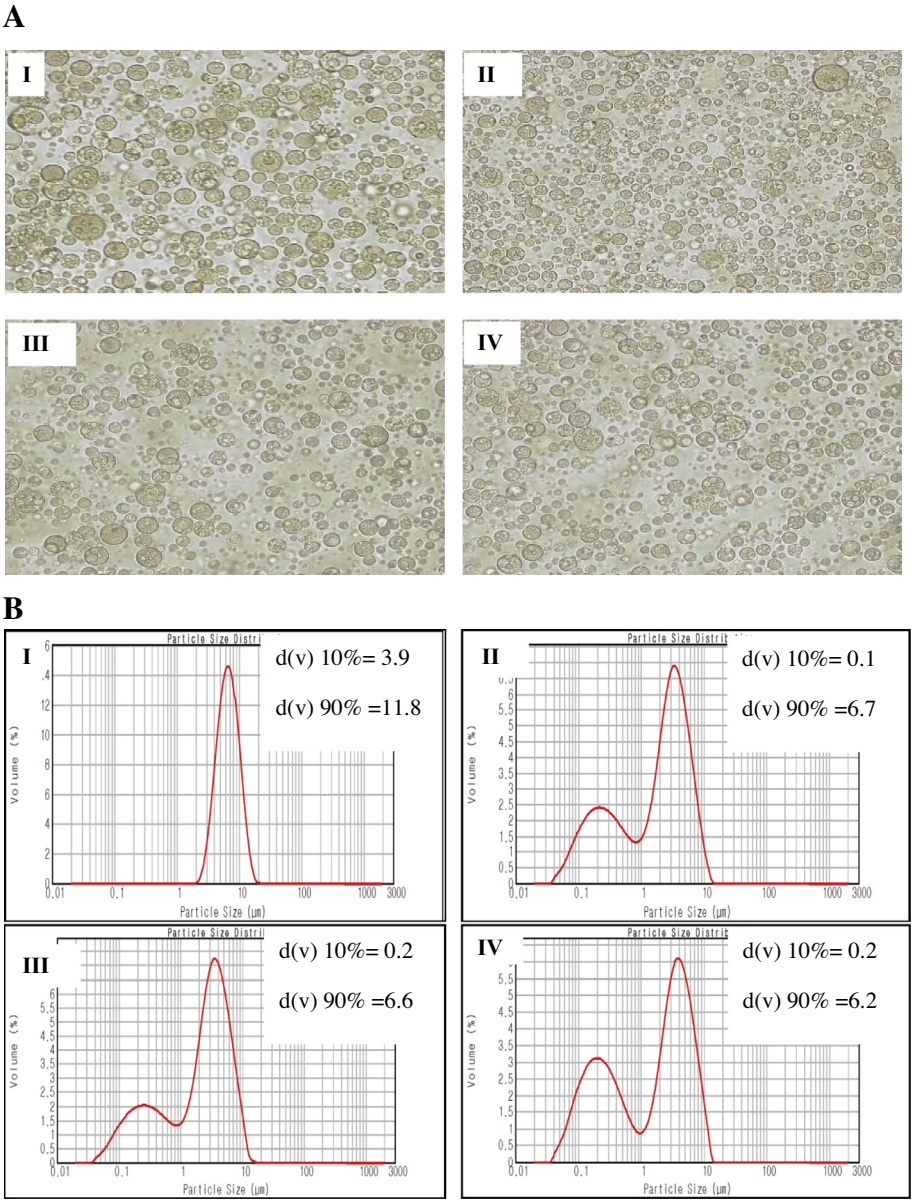

Fig. 1. (A) Micrographs of water-in-oil-in-water double emulsions (I) blank; (II) curcumin; (III) catechin and (IV) curcumin and catechin co-loaded W/O/W emulsion. (B) Typical droplet size distribution of W/O/W emulsions prepared with PGPR (6%) and 1% Tween 80. (I) blank; (II) curcumin; (III) catechin; (IV) curcumin and catechin co-loaded W/O/W emulsion. (C) Graphical representation of blank and curcumin and catechin co-loaded emulsion.

Table 2
The droplet size distribution and zeta potential of W/O/W emulsions stabilised with 1% Tween 80 (25 vol% primary emulsion) with and without curcumin and/or catechin at different time intervals.

Time elapsed	Physicochemical quantity	Droplet properties for different core deliveries			
		Blank	Curcumin	Catechin	Curcumin catechin
Day 0	d_{32} (μm)	0.59 ± 0.4	0.46 ± 0.1	0.53 ± 0.1	0.47 ± 0.2
	d_{43} (μm)	3.88 ± 0.3	2.88 ± 0.3	2.82 ± 0.2	3.06 ± 0.5
	$d(v)$ 90% (μm)	12.1 ± 2.3	6.6 ± 0.3	8.0 ± 2.4	6.7 ± 0.1
	Zeta potential (mV)	−20 ± 2.8	−17 ± 0.7	−18 ± 2.6	−18 ± 0.9
Day 7	d_{32} (μm)	0.64 ± 0.6	0.55 ± 0.3	0.58 ± 0.4	0.43 ± 0.1
	d_{43} (μm)	3.66 ± 0.7	3.4 ± 1.1	2.6 ± 0.8	3.6 ± 0.3
	d(v) 90% (μm)	10.5 ± 3.4	6.7 ± 1.0	9.7 ± 1.0	6.6 ± 0.7
Day 15	d_{32} (μm)	0.87 ± 0.5	0.49 ± 04	0.50 ± 0.4	0.52 ± 0.2
	d_{43} (μm)	3.98 ± 0.3	3.0 ± 0.2	3.6 ± 0.5	3.1 ± 0.9
	d(v) 90% (μm)	9.9 ± 1.6	6.0 ± 1.3	8.5 ± 0.8	6.2 ± 1.1

$d(v)$ 10% was ≈4 μm for blank emulsions whereas, in catechin- and/or curcumin-loaded emulsions, it was ≈0.2 μm, which showed the presence of much smaller droplets after the encapsulation of curcumin and catechin. This resulted in conversion of the unimodal size distribution observed in the blank emulsion to a bimodal size distribution in curcumin- and/or catechin-loaded emulsions. This reduction in size may have been due to the surface activity of polyphenols. Recent studies have shown that flavonoids, such as catechin, rutin and quercetin, can decrease the interfacial tension at the oil–water interface, which might be the reason for the decreased size of the double emulsion after encapsulation with curcumin and/or catechin (Akhtar, Murray, Afeisume, & Khew, 2014; Luo et al., 2012). This is an important observation because successes in using nutraceuticals as emulsifiers reduce the dependency on synthetic surfactants, and they act as edible Pickering particles for stabilisation of double emulsions. Fabricated double emulsions were stable for a study period of 15 days in which noticeable increases in d_{32} and d_{43} were not observed.

The zeta potential of all double emulsions (irrespective of curcumin and/or catechin loading) was approximately −20 mV, which is known to be sufficient to produce stable emulsions (Zimmermann & Müller, 2001). Our aim was to deliver curcumin and catechin to the intestine to increase their stability and bioaccessibility by micelle formation. Hence, Tween 80 was selected as a hydrophilic surfactant known to protect lipid nanoparticles from degradation in the stomach due to acidic conditions, the action of pepsin, and lipid oxidation, due to its molecular structure (Aditya et al., 2013; Kenmogne-Domguia, Moisan, Viau, Genot, & Meynier, 2014).

3.2. Encapsulation efficiency

High encapsulation efficiency was observed for curcumin and catechin (Fig. 2). Encapsulation efficiency was 88 ± 2% for curcumin and 97 ± 0.3% for catechin. In the case of co-loaded nanoparticles, no significant difference in encapsulation efficiency of curcumin and catechin was observed in comparison with a single nutraceutical-loaded emulsion. This high encapsulation efficiency may have been due to the hydrophobic environment for the hydrophobic nutraceutical molecule curcumin and hydrophilic environment for the hydrophilic molecule catechin.

Another important observation was total loading efficiency which can be described as the total amount of bioactives or molecules that remained entrapped in a known amount of the emulsion sample. In the single nutraceutical-loaded emulsion, it was 0.1% for curcumin and 0.075% for catechin. However, in the co-loaded emulsion, the loading efficiency was 0.175%, which was significantly higher ($P < 0.05$) than its counterpart single nutraceutical-loaded emulsion. This further shows that, by encapsulation, we can deliver more nutraceuticals without increasing carrier materials. Requirements for a higher amount of carrier material to deliver a smaller amount of nutraceutical that may alter organoleptic properties and toxicity profiles is the major hurdle in bringing nanostructured food matrices into the market.

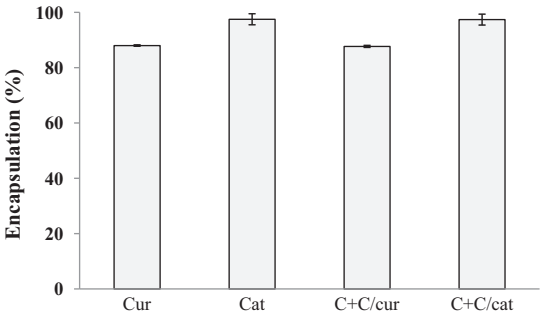

Fig. 2. Encapsulation efficiencies of double emulsions stabilised with hydrophobic stabilizer PGPR and hydrophilic stabilizer Tween 80. Cur: curcumin emulsion; Cat: catechin emulsion; C+C/cur: curcumin in curcumin and catechin co-loaded emulsion; C+C/cat: catechin in curcumin and catechin co-loaded emulsion (mean ± SD; $n = 3$).

3.3. In vitro release and stability study in SGF

The release of curcumin and catechin from the W/O/W double emulsion was tested *in vitro* in enzyme-free SIM. Ethanol (40%) was used to provide the sink condition for curcumin and stability for catechin. Controlled release was observed for curcumin from curcumin alone and co-loaded emulsions (Fig. 3A). Over 360 min, ≈46–50% of curcumin was released. In the catechin-loaded double emulsion, burst release (immediate rush of bioactives or encapsulated molecules from the core of the container to the releasing media) was observed whereby ≈30% of catechin was released within 30 min and >45% within 1 h. At the end of the study, ≈90% of catechin was released from catechin-loaded and co-loaded double emulsions. The overall released amount and rate of release was higher for catechin compared with curcumin. The main reason may be the hydrophilic nature of catechin ($\log P = 0.80$) (Hatzidimitriou, Nenadis, & Tsimidou, 2007), which enables the molecule to diffuse readily into hydrophilic-releasing media whereas curcumin, which is hydrophobic ($\log P = 3.1$) (Aditya et al., 2013), tends to remain within lipid molecules. Co-loading of curcumin and catechin had no significant effect on each other's release.

Low stability in the GIT is a major hurdle for the bioavailability of curcumin and catechin. Curcumin and catechin degrade rapidly

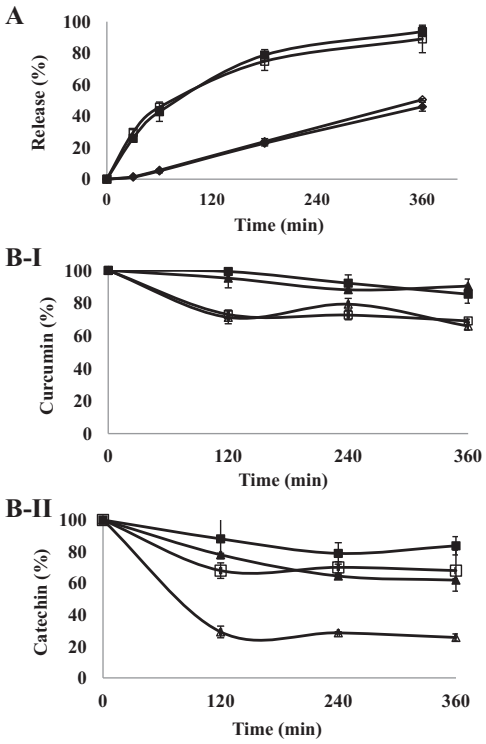

Fig. 3. (A) *In vitro* release study of curcumin and catechin in enzyme free SIF. Ethanol (40%) was used to provide the sink condition for curcumin and stability for catechin. Closed square: catechin release from curcumin and catechin co-loaded emulsion; open square: catechin release from catechin emulsion; closed triangle: curcumin release from curcumin and catechin co-loaded emulsion; open square: curcumin release from curcumin emulsion. (B) Stability of (B-I) curcumin and (B-II) catechin after incubation in enzyme free simulated gastrointestinal medium (SIF and SGF). Closed square: curcumin or catechin emulsion incubated in SGF; open square: curcumin or catechin emulsion in SIF: closed triangle: free curcumin or catechin incubated in SGF; open triangle: free curcumin or catechin incubated in SIF (mean ± S.D.; $n = 3$).

in neutral or alkaline conditions compared with acidic conditions (Green, Murphy, Schulz, Watkins, & Ferruzzi, 2007; Noack, Oidtmann, Kutza, & Mader, 2012). We hypothesised that the slow-releasing nature of curcumin and, to some extent, catechin, helps to protect them from degradation in the GIT. To further evaluate this hypothesis, a stability study was conducted. Curcumin entrapped in an emulsion and free curcumin showed >85% stability in SGF (Fig. 3B-I), whereas its stability fell to ≈65–70% in SIF after incubation for 360 min. This finding was in agreement with studies showing faster degradation of curcumin in alkaline intestinal conditions (pH ⩾ 7.0) compared with acidic gastric conditions (Noack et al., 2012). With regards to catechin (Fig. 3B-II), ≈83% of catechin was stable in SGF after incubation for 360 min if added as an emulsion, whereas its stability decreased significantly to ≈60% if added as free molecules. In SIF, catechin degradation was faster and ≈35% catechin degraded if added as an emulsion and ≈74% degradation was observed for free catechin. The vulnerability of catechin in alkaline conditions, specifically in SIF, has been postulated to be due to auto-oxidation of 3′, 4′, and 5′ hydroxyl groups (Record & Lane, 2001; Zhu, Zhang, Tsang, Huang, & Chen, 1997). Interestingly, even though more catechin was degraded in SIF compared with SGF in all forms, catechin addition in the form of an emulsion significantly increased their stability. The enhanced stability of catechin in SIF if added as an emulsion may be due to the: (a) formation of a protein (gelatin)–polyphenol (catechin) complex and the presence of ascorbic acid (which is known to stabilise catechin by increasing its water solubility and by quenching the free radicals formed under neutral or alkaline pH) (Mahmoud, Chedea, Detsi, & Kefalas, 2013); (b) controlled-release property of W/O/W emulsions, which protect catechin from degradation until it is released from the inner aqueous phase (and which might also contribute to its their enhanced stability) (Green et al., 2007; Proniuk, Liederer, & Blanchard, 2002). Once again, these findings demonstrated the importance of formulation excipients and controlled release on the stability of catechin in the GIT, which has been observed previously (Ortega, Reguant, Romero, Macia, & Motilva, 2009).

3.4. In vitro bioaccessibility study

This study was conducted to ascertain the effect of the encapsulation of curcumin and catechin on their solubilisation in SGF and thereby their bioaccessibility. Hydrophobic curcumin and hydrophilic catechin are known to have poor cell permeability (Chung et al., 2013; Wahlang, Pawar, & Bansal, 2011). The bioaccessibility of curcumin was ≈72% in a curcumin-loaded emulsion and was 68% after encapsulation in a co-loaded emulsion (Fig. 4). However, the bioaccessibility of curcumin fell significantly to ≈16% if added as a solution, which shows the poor bioavailability of curcumin. This increased bioaccessibility may be due to the presence of micellar structures to accommodate curcumin within their core. They were formed after lipid digestion by lipase and pancreatin, and were assisted by other excipients in the SIF. Previous studies by our research team and others have shown similar increases in the bioaccessibility of curcumin (Aditya et al., 2013; Ahmed, Li, McClements, & Xiao, 2012; Koga et al., 2010). The bioaccessible fraction was ≈60% and ≈54% for catechin-loaded and co-loaded emulsions, respectively, but fell significantly to ≈10% if catechin was added as a free molecule. The reason for the low bioaccessibility of free catechin may be due to its notoriously low stability in SIF (pH > 6) (Chung et al., 2013). The increase in its bioaccessibility in emulsions may be due to three reasons. The first is the stability of the W/O/W emulsion in SGF due to Tween 80 acting as a hydrophilic stabilizer that protects the carrier from degradation in acidic environments and from the action of pepsin due to its molecular structure (van Aken, Bomhof, Zoet, Verbeek, & Oosterveld, 2011). These actions stop catechin degradation in SGF by keeping it within the inner aqueous phase. However, in free solution, some degradation in SGF occurs, as seen in our stability study (Section 3.4). The second reason is the interaction of lipid molecules with catechin, which results in their entrapment within formed micellar structures and protects them from degradation. Finally, due to the presence of antioxidants, such as ascorbic acid, curcumin might also protect catechin molecules from degradation in alkaline SIF.

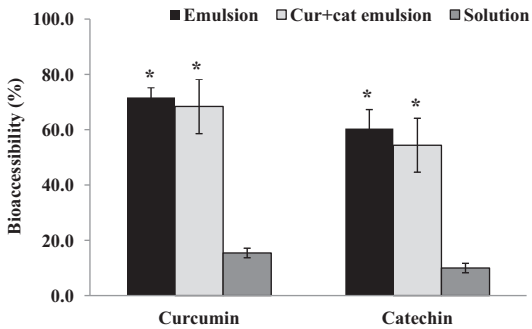

Fig. 4. Bioaccessible fraction of curcumin and/or catechin in the supernatant after the simulated intestinal digestion of respective nutraceuticals. Emulsion: either curcumin or catechin loaded W/O/W emulsion; co-loaded emulsion: both curcumin and catechin loaded W/O/W emulsion; free nutraceuticals: both curcumin and catechin co-dispersed solution (mean ± S.D.; $n = 3$).

In addition, these excipients increase the solubility of catechin in water, thereby facilitating its incorporation within micelles in a more efficient way (Green et al., 2007; Peters, Green, Janle, & Ferruzzi, 2010). Studies have shown similar increased stability and bioaccessibility of polyphenols if used with fat materials (Ortega et al., 2009). Co-loading of curcumin and catechin has no adverse effect on each other's bioaccessibility. This clearly demonstrates the importance and benefit of encapsulating them in emulsions (particularly co-loading curcumin and catechin).

4. Conclusion

Double emulsion entrapment of hydrophilic catechin and hydrophobic curcumin was fabricated using a two-step emulsification method. The fabricated system showed a synergistic effect between the components. Encapsulation of curcumin and catechin resulted in an increase in their stability and bioaccessibility. The presence of catechin and curcumin helped to reduce the size of the emulsion.

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