Food Chemistry 214 (2017) 366–373

Contents lists available at ScienceDirect

Food Chemistry

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

Analytical Methods

Facile preparation of water soluble curcuminoids extracted from turmeric (*Curcuma longa* L.) powder by using steviol glucosides

Thi Thanh Hanh Nguyen [a], Jinbeom Si [a], Choongil Kang [b], Byoungsang Chung [b], Donghwa Chung [a,c], Doman Kim [a,c,*]

[a] The Institute of Food Industrialization, Institutes of Green Bio Science & Technology, Seoul National University, Pyeongchang-gun, Gangwon-do 25354, South Korea
[b] OTTOGI Corporation, Anyang, Kyunggi 06177, South Korea
[c] Graduate School of International Agricultural Technology, Seoul National University, Pyeongchang-gun, Gangwon-do 25354, South Korea

ARTICLE INFO

Article history:
Received 9 April 2016
Received in revised form 18 June 2016
Accepted 14 July 2016
Available online 15 July 2016

Keywords:
Turmeric
Curcuminoids
Solubilization
Stevioside
Steviol glucoside
Rebaudioside A

ABSTRACT

Curcuminoids from rhizomes of *Curcuma longa* possess various biological activities. However, low aqueous solubility and consequent poor bioavailability of curcuminoids are major limitations to their use. In this study, curcuminoids extracted from turmeric powder using stevioside (Ste), rebaudioside A (RebA), or steviol glucosides (SG) were solubilized in water. The optimum extraction condition by Ste, RebA, or SG resulted in 11.3, 9.7, or 6.7 mg/ml water soluble curcuminoids. Curcuminoids solubilized in water showed 80% stability at pH from 6.0 to 10.0 after 1 week of storage at 25 °C. The particle sizes of curcuminoids prepared with Ste, RebA, and SG were 110.8, 95.7, and 32.7 nm, respectively. The water soluble turmeric extracts prepared with Ste, RebA, and SG showed the 2,2-diphenyl-1-picrylhydrazyl radical scavenging (SC_{50}) activities of 127.6, 105.4, and 109.8 μg/ml, and the inhibition activities (IC_{50}) against $NS2B\text{-}NS3^{pro}$ from dengue virus type IV of 14.1, 24.0 and 15.3 μg/ml, respectively.

1. Introduction

Turmeric (*Curcuma longa* L.) belongs to the *Zingiberaceae* family. It is distributed throughout tropical and subtropical regions of the world, and wildly cultivated in Southeast Asia (Goel, Kunnumakkara, & Aggarwal, 2008). Turmeric contains important analogues, curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) as main components. It also contains 14 terpene-conjugated curcuminoids (Anand et al., 2008; Lin et al., 2013). Depending on its origin and soil conditions, turmeric contains 2–9% (w/w) curcuminoids (Priyadarsini, 2014). Curcumin is the most abundant curcuminoid in turmeric (Anand et al., 2008). Turmeric has been used for food flavouring, colour, and as a component in traditional medicine. In preclinical studies, curcuminoids have activities, such as scavengers of free radicals and reactive oxygen species (ROS) (Ahsan, Parveen, Khan, & Hadi, 1999), antidiabetic by suppressing blood glucose level (Nishiyama et al., 2005), or inhibit the proliferation of a wide variety of tumor cells, including leukemia, lung cancer, head & neck cancer, pancreatic cancer, breast cancer, and prostate cancer (Sandur et al., 2007). Although curcuminoids are extremely safe, tolerable, and nontoxic in animal and human studies even at very high doses (⩽12 g per day) (Cheng et al., 2001; Lao et al., 2006; Shoba et al., 1998), they are not approved as therapeutic agents because of their limited solubility in aqueous environments, such as the human gastrointestinal tract and limited gastrointestinal absorption (Anand et al., 2008), rapid metabolism both in the intestines and the liver (Ireson et al., 2002), chemical instability in alkaline medium (Wang et al., 1997), and inability to reach the blood in concentrations necessary to affect disease markers or clinical end points, even at chronic doses of up to 12 g a day (Lao et al., 2006). For example, curcumin was reported to reverse disease states at concentrations of 12.9 μg/ml for human colon cancer cell (Collett & Campbell, 2004), 35.1 μg/ml for radical scavenging activity (Somparn, Phisalaphong, Nakornchai, Unchern, & Morales, 2007), 9.2 μg/ml for human pancreatic alpha amylase (Ponnusamy, Zinjarde, Bhargava, Rajamohanan, & Ravikumar, 2012). However, curcumin is undetectable or extremely low (6×10^{-3} μg/ml at 1 h in serum level) with an intake dose of 2 g in humans (Shoba et al., 1998). Even after a high dose intake of 4, 6, and 8 g of curcumin daily for three months, serum curcumin concentration was only 1.9×10^{-1}, 2.3×10^{-1}, and 6.5×10^{-1} μg/ml, respectively (Cheng et al., 2001). To overcome the low bioavailability, increasing the serum levels of curcuminoids using liposomes, polymeric micelles,

* Corresponding author at: The Institute of Food Industrialization, Institutes of Green Bio Science & Technology, Seoul National University, Pyeongchang-gun, Gangwon-do 25354, South Korea.
E-mail addresses: hara2910@snu.ac.kr (T.T.H. Nguyen), tlwlsqja88@snu.ac.kr (J. Si), gilkang@ottogi.co.kr (C. Kang), bschung@ottogi.co.kr (B. Chung), dchung@snu.ac.kr (D. Chung), kimdm@snu.ac.kr (D. Kim).

http://dx.doi.org/10.1016/j.foodchem.2016.07.102
0308-8146/

phospholipids, nanoparticle based drug delivery systems (Kanai et al., 2012; Letchford, Liggins, & Burt, 2008; Li, Braiteh, & Kurzrock, 2005; Liu, Lou, Zhao, & Fan, 2006), and the development of new curcumin analogues (Ohori et al., 2006) have been investigated to improve the water solubility as well as oral bioavailability of curcumin. By increasing the water solubility of curcumin, oral administration was demonstrated to yield more than 30-fold higher bioavailability compared with conventional curcumin in rat models (Sasaki et al., 2011). In healthy human subjects, the maximum serum curcuminoid concentration after administration of 30 mg of water soluble curcuminoids was 30 ng/ml compared to 2 ng/ml after conventional curcumin administration (Sasaki et al., 2011). Although curcuminoids can be chemically synthesized, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) specifications allow only curcuminoids to be extracted from natural source material to be used as food additives.

Several terpene glycosides, such as mogroside V, paenoiflorin, geniposide, rubusoside (Ru), stevioside (Ste), rebaudioside (RebA), and steviol monoside, have shown the ability to enhance the solubility of a number of pharmaceutically and medically important compounds with poor solubility in water (Nguyen et al., 2014; Zhang et al., 2011). Steviol glucosides, such as Ru, Ste, and RebA, are the main sweet components of *Rubus suavissimus* S. Lee (Rosaceae) and *Stevia rebaudiana* Bertoni leaves (Upreti, Strassburger, Chen, Wu, & Prakash, 2011). Ru can enhance the solubility of curcumin from 0.6 mg/ml to 2.3 mg/ml with 1–10% Ru (w/v) solution in water (Zhang et al., 2011). Although Nguyen et al. have developed a facile enzymatic process for preparing Ru from Ste (Nguyen et al., 2014), currently Ste and RebA are commercially available. They have been approved by the Food and Drug Administration to be used in food application.

In this study, water soluble curcuminoids were directly prepared from turmeric powder (*Curcuma longa* L.) by using Ste, RebA, or SG along with the characterization of their physical and biological functionality.

2. Materials and methods

2.1. Preparation of water soluble curcuminoids from turmeric powder by using Ste, Reb, or SG

Turmeric powder was purchased from a local market. Curcuminoids (⩾94%) containing ⩾80% curcumin (PubChem CID: 969516), DMC (PubChem CID: 5469424), and BDMC (PubChem ID: 5315472) were purchased from Sigma as standard. Ste, RebA (PubChem CID: 6918840), and SG with α-1-4 linkages were provided by Daepyung Co., Ltd (Gyeonggi-do, Korea). Turmeric-stevioside (turmeric powder treated with Ste to solubilize curcuminoids; Tum-Ste), turmeric-rebaudioside A (turmeric powder treated with RebA to solubilize curcuminoids; Tum-Reb), or turmeric-stevioside glucosides (turmeric powder treated with SG to solubilize curcuminoids; Tum-SG) solution was prepared as reported previously (Nguyen et al., 2014). The effect of water concentration to extract curcuminoids was investigated. Each Ste, RebA, or SG at 10% (w/v) was mixed with 30% (w/v) of turmeric powder followed by addition of 0–80% (v/v) of water in ethanol. The mixture in ethanol solution was vortexed for 15 min and centrifuged at 12,000×*g* for 10 min. The supernatant was transferred to a new tube and ethanol was evaporated (Nguyen et al., 2014). The resulting powders were dissolved in water, centrifuged at 12,000×*g* for 10 min, and filtered through 0.20 μm membrane (Agilent, Santa Clara, CA, USA). Thin layer chromatography (TLC) with an ascent of acetonitrile/water 85:15 (v/v) for curcuminoids and chloroform/methanol 19:1 (v/v) was used to determine curcuminoids. Curcuminoids on silica gel 60F_{254}TLC plate (Merck, Darmstadt, Germany) were visualized under UV_{254nm}. They were also visualized by dipping the TLC plate into a solvent mixture of 0.5 (w/v) N-(1-naphthyl)ethylenediamine dihydrochloride and 5% (w/v) sulfuric acid in methanol followed by heating at 121 °C for 3 min. The residual amount of curcuminoids was determined using SpectraMax M3 (Molecular Devices, Sunnyvale, CA, USA) at 425 nm (Gopal, Muthu, & Chun, 2015). Water soluble curcuminoids yields were calculated using the following equation:

$$\text{Water soluble curcuminoids yield}(\%) = \frac{\text{Curcuminoid extracted}(g)}{\text{Tumeric powder used}(g)} \times 100$$

The effect of Ste, RebA, or SG concentration to enhance the solubility of curcuminoids from turmeric powder was studied with different concentration of Ste, RebA, or SG (from 0.5 to 10%, w/v) mixing with 30% (w/v) of turmeric powder in 100% (v/v) ethanol for Ste or in 10% (v/v) of water in ethanol for RebA or SG.

The effect of turmeric concentration to enhance the yield of soluble curcuminoids from turmeric power using 8% (w/v) of Ste, RebA or SG (100% (v/v) ethanol for Ste, 10% (v/v) of water in ethanol for RebA or SG) was studied at different concentrations of turmeric powder (from 10 to 40%, w/v). The pH of curcuminoids in water after extraction was determined by direct measurement with a pH meter (Thermo Scientific, Waltham, MA, USA). Water soluble curcuminoids were freeze dried and stored at −20 °C for further study.

The ratio of curcumin, DMC, and BDMC in the water soluble turmeric extract by Ste, RebA, and SG was analyzed using integrated density values (IDV) by employing the AlphaEaseFC 4.0 program (Alpha Inotech, San Leandro, CA, USA) with curcumin, DMC, and BDMC as the standards.

2.2. pH stability of water soluble curcuminoids

For pH stability of water soluble curcuminoids extracted from turmeric powder, several pH conditions (50 mM Na-P pH 6.0, pH 7.0, pH 7.5, NaOH-glycin pH 8.0 and pH 10.0) were evaluated. Two micrograms of curcuminoids were dissolved in buffer and kept at 25 °C for 1 week. The samples were then centrifuged at 12,000×*g* for 10 min and filtered through 0.20 μm membrane. The residual amount of curcuminoids was determined using spectrophotometry at 425 nm and TLC method as described previously (Chen et al., 2015).

2.3. Particle size analysis

The samples were prepared by taking 10 mg of the lyophilized of Ste, RebA, SG, Tum-Ste, Tum-RebA and Tum-SG in 10 ml of distilled water or 20 mg of Tum-Ethanol in ethanol. Dynamic light scattering (DLS) was performed to measure the size of Ste, RebA, SG, water soluble turmeric extracts by Ste, RebA, and SG in solution with a zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, UK) (Murdock, Braydich-Stolle, Schrand, Schlager, & Hussain, 2008) at 25 °C. Each sample was measured in triplicated and the average values were used.

2.4. Antioxidant effects

The antioxidant activities of water soluble turmeric extracts were evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method as described previously. Samples were dissolved in water. Each sample was mixed with 100 μM (DPPH) in methanol solution to give a final concentration of 10–500 μg of water soluble turmeric extract by Ste, Re, or SG per ml. After 30 min of incubation at 25 °C in total darkness, the absorbance of each mixture was measured at 517 nm on a microplate reader (Molecular Devices, Sunnyvale, CA, USA). A negative control

(containing all reagents except the test sample) was used for this test. Curcuminoids (⩾94%) containing ⩾80% curcumin and α-tocopherol, and trolox were used as the positive controls. DPPH radical-scavenging activity was converted into percentage of antioxidant activity using the following equation:

$$\text{DPPH radical-scavenging activity}(\%) = \frac{(\text{Absorbance of control} - \text{Absorbance of test sample})}{\text{Absorbance of control}} \times 100$$

A linear regression curve was established in order to determine the SC_{50} value, i.e., the amount of sample necessary to decrease 50% of the absorbance of DPPH. All analyses were carried out in triplicate. Results were expressed as mean ± standard error (SEM).

2.5. Inhibitory activity of curcuminoids against DENV4 NS2B-NS3^{pro}

Recombinant DENV4 NS2B-NS3^{pro} enzyme was prepared as described previously (Nguyen et al., 2013). Water soluble turmeric extract was dissolved in water and tested in triplicate at a concentration of 1–200 μg/ml to determine its ability to inhibit NS2B-NS3^{pro}. Assays were performed in a reaction mixture (final volume 100 μl) containing 0.04 U enzymes, 1.65 μM fluorogenic tetrapeptide substrate, benzoyl-norleucine-lysine-arginine-arginine-7-amino-4-methyl coumarin (AMC) (Bachem, Bubendorf, Switzerland), 2 μl of inhibitor, and 40 mM Tris buffer (pH 7.5). The reaction was measured in a SpectraMax Gemini XPS apparatus (Molecular Devices, Sunnyvale, CA, USA) (λex = 380 nm, λem = 460 nm) at 37 °C for 20 min. Curcuminoids (⩾94%) containing ⩾80% curcumin was used as the positive control. The inhibition was calculated using the following equation:

$$\text{Remaining activity}(\%) = [(S - So)/(C - Co)] \times 100$$

where C was the fluorescence of the control (enzyme, buffer, and substrate) after 20 min of incubation, C_o was the fluorescence of the control at time zero, S was the fluorescence of the tested sample (enzyme, sample solution, and substrate) after incubation, and S_o was the fluorescence of the tested sample at time zero. The 50% inhibitory concentration (IC_{50}) was defined as the concentration of NS2B-NS3^{pro} inhibitor necessary to reduce NS2B-NS3^{pro} activity by 50% relative to a reaction mixture containing NS2B-NS3^{pro} enzyme without inhibitor.

2.6. Statistical analysis

The results of turmeric extraction using Ste, RebA, and SG were expressed as mean ± SEM of three identical experiments. One-way analysis of variance (ANOVA) was performed using GraphPad Prism software version 5.0 (GraphPad Software Inc., San Diego, CA, USA) with Tukey's multiple comparison test. Statistical significance was determined at $p \leqslant 0.05$.

3. Results

3.1. Extraction of water soluble curcuminoids from turmeric powder

The curcuminoids powder prepared with ethanol directly from turmeric powder showed low or no solubility in water (Fig. 1A).

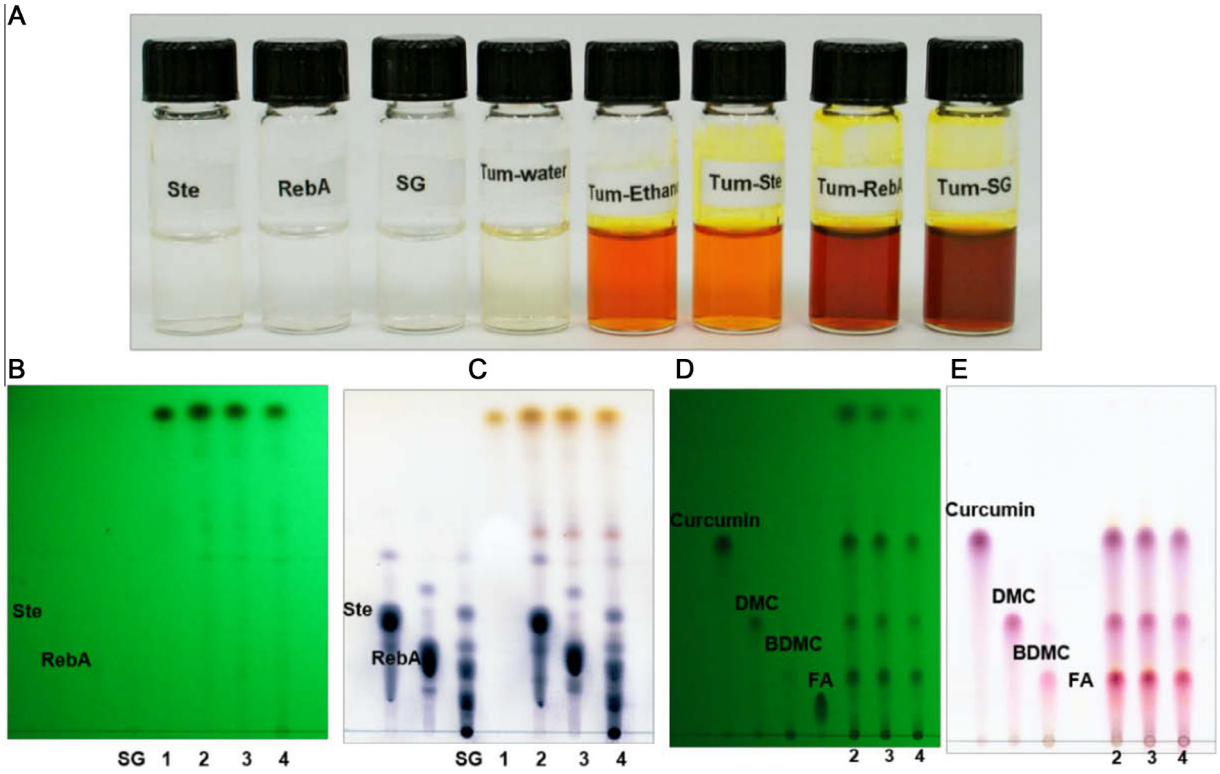

Fig. 1. Turmeric extracts prepared with stevioside, rebaudioside A, or steviol glucosides. A: Solubility of curcuminoids in water solution with 10% (w/v) Ste, 10% (w/v) RebA, and 10% (w/v) SG. Tum-water: curcuminoids was extracted from turmeric powder using water. Tum-Ethanol: curcuminoids was extracted from turmeric powder using ethanol. Tume-Ste: curcuminoids was extracted from turmeric powder using Ste ethanol solution and ethanol was removed by evaporation and re-dissoluble in water. Tum-RebA: curcuminoids was extracted from turmeric powder using rebaudioside A ethanol solution and ethanol was removed by evaporation and re-dissoluble in water. Tume-SG: curcuminoids was extracted from turmeric powder using steviol glucosides ethanol solution and ethanol was removed by evaporation and re-dissoluble in water. B: Lane Ste: 10% (w/v) of stevioside standard, lane RebA: 10% (w/v) of rebaudioside A standard, lane SG: 10% (w/v) of steviol glucosides standard, Lane DMC: 0.5% (w/v) of demethoxycurcumin in ethanol; Lane BDMC: 1% (w/v) of bisdemethoxycurcumin in ethanol; Lane FA: 0.5% ferulic acid in ethanol; lane 1: curcuminoids standard from Sigma, lane 2–4: water soluble curcuminoids prepared using Ste, RebA, or SG. Thin layer chromatography analysis of curcuminoids with one ascent of acetonitrile/water 85:15 (v/v) and chloroform/methanol 19:1 (v/v). They were detected under UV_{254nm} (B, D) and by dipping the TLC plate into a solvent mixture of 0.5 (w/v) N-(1-Naphthy) ethylenediamine dihydrochloride and 5% (w/v) sulfuric acid in methanol followed by heating at 90 °C for 3 min (C, E).

The turmeric powder treated with ethanol had yellow colour. After solubilized in water, it showed a clear supernatant without yellow colour. However, curcuminoids extracted directly from turmeric powder using Ste, RebA, or SG resulted in clear and yellow colour in water (Fig. 1A). These curcuminoids in water were confirmed by using TLC analysis under UV_{254nm} (Fig. 1B) and by using developing solution (Fig. 1C). Three main analogues of curcuminoids (curcumin, DMC, and BDMC) were detected on TLC as shown in Fig. 1D (under UV_{254nm}) and Fig. 1E. The R_f values for curcumin, DMC, and BDMC were 0.57, 0.41, and 0.29, respectively. Thus, Ste, RebA or SG could be used to prepare water soluble curcuminoids directly from turmeric powder. The pH values of solubilized curcuminoids prepared with Ste, RebA, and SG were 4.7, 5.0, and 4.9, respectively.

3.2. Concentration effect of water, Ste, RebA, SG, and turmeric powder on curcuminoids yields

When Ste was used to extract curcuminoids from turmeric powder, as water concentration with Ste increased, the amount of prepared water soluble curcuminoids was reduced from 33.3 ± 1.3 to 14.6 ± 0.7 mg per gram of turmeric powder (Table 1). The highest water soluble curcuminoids yield (33.3 ± 1.3 mg/g turmeric powder) was obtained with 100% (v/v) ethanol (without adding water) with Ste. However, both RebA and SG showed the highest yields of water soluble curcuminoids with 10% (v/v) of water in ethanol. The yields were 26.8 ± 1.6 mg curcuminoids/g turmeric powder with RebA and 23.1 ± 1.6 mg curcuminoids/g turmeric powder with SG. The effect of different concentrations of Ste, RebA, or SG on the yields of soluble curcuminoids was also investigated. As the concentration of Ste, RebA, or SG was increased from 0.5% to 8% (w/v), the amount of water soluble curcuminoids was also increased from 0.2 to 34.5 ± 1.4 mg/g with Ste, from 0.04 to 32.4 ± 0.2 mg/g with RebA, and from 0.1 to 21.1 ± 0.3 mg/g with SG (Table 2). The highest concentration of Ste, RebA, or SG to obtain water soluble curcuminoids was 8% (w/v). In case of turmeric powder, the yield of water soluble curcuminoids decreased as the amount of turmeric powder was increased (Table. 3). As the amount of turmeric powder was increased from 1% (w/v) to 40% (w/v), the yields of water soluble curcuminoids was decreased

Table 1
Concentration of water soluble curcuminoids obtained from turmeric powder by using stevioside, rebaudioside, or steviol glucosides.

Water added during turmeric extraction process (%, v/v)	Soluble curcuminoids with Ste (mg/g turmeric powder)	Soluble curcuminoids with RebA (mg/g turmeric powder)	Soluble curcuminoids with SG (mg/g turmeric powder)
0	33.4 ± 1.3[a]	17.0 ± 1.2[a]	18.1 ± 0.6[a]
10	30.0 ± 2.5	26.8 ± 1.6	23.1 ± 1.6
20	29.8 ± 2.0	25.5 ± 1.0	23.0 ± 1.8
30	28.9 ± 1.7[b]	24.3 ± 1.6[b]	17.3 ± 0.8[b]
40	25.1 ± 2.5[c]	19.7 ± 0.6[c]	16.2 ± 0.6[c]
50	24.0 ± 1.4[d]	18.1 ± 1.7[d]	16.1 ± 0.8[d]
60	19.2 ± 0.9[e]	11.2 ± 1.2[e]	14.2 ± 0.5[e]
80	14.6 ± 0.7[f]	13.5 ± 0.6[f]	8.7 ± 0.2[f]

Each value is mean ± SEM of three identical experiments; mean values with the same row sharing the same alphabetical letter superscripts are statistically significant at $P \leqslant 0.05$.

Table 2
Effect of stevioside, rebaudioside A, or steviol glucosides concentration on the yield of water soluble curcuminoids from turmeric powder.

Ste, RebA, SG concentration (%, w/v)	Soluble curcuminoids with Ste (mg/g turmeric powder)	Soluble curcuminoids with RebA (mg/g turmeric powder)	Soluble curcuminoids with SG (mg/g turmeric powder)
0.5	0.2[a]	0.04[a]	0.1[a]
1	0.8[b]	1.7[b]	0.4[b]
2	6.6 ± 0.1[c]	6.2 ± 0.1 [c]	2.8 ± 0.1 [c]
3	14.0 ± 0.3[d]	12.1 ± 0.1[d]	6.4 ± 0.2[d]
4	20.0 ± 0.5[e]	17.0 ± 0.4[e]	9.0 ± 0.1[e]
5	28.1 ± 0.7[f]	20.9 ± 0.2 [f]	12.5 ± 0.3[f]
6	28.7 ± 0.4[g]	24.5 ± 0.4[g]	16.5 ± 0.6[g]
8	34.5 ± 0.6[h]	32.4 ± 0.1[h]	21.1 ± 0.3[h]
10	29.7[i]	26.4 ± 0.6[i]	19.1 ± 0.1[i]

Each value is mean ± SEM of three identical experiments; mean values with the same row sharing the same alphabetical letter superscripts are statistically significant at $P \leqslant 0.05$.

Table 3
Effect of turmeric powder concentration on the yield of water soluble curcuminoids from turmeric powder.

Turmeric concentration (%, w/v)	Soluble curcuminoids with Ste		Soluble curcuminoids with RebA		Soluble curcuminoids with SG	
	mg/g turmeric powder	mg/ml	mg/g turmeric powder	mg/ml	mg/g turmeric powder	mg/ml
1	69.3 ± 4.0	0.7	70.6 ± 0.6	0.7	73.1 ± 1.0	0.7
3	56.2 ± 1.3	1.7[a]	65.1 ± 0.4	2.0[a]	65.8 ± 1.0	2.0[a]
5	51.2 ± 3.7	2.6 ± 0.2[b]	60.3 ± 0.9	3.0[b]	61.3 ± 0.5	3.1[b]
7.5	50.4 ± 0.2	4.3	48.4 ± 0.4	3.6	50.7 ± 0.7	3.8 ± 0.1
12.5	48.7 ± 0.5	6.1 ± 01[c]	48.3 ± 0.6	6.0 ± 0.1[c]	38.1 ± 0.6	4.8 ± 0.1[c]
15	47.0 ± 3.2	7.0 ± 0.6[d]	47.5 ± 0.2	7.1[d]	37.3 ± 1.3	5.7 ± 0.2[d]
17.5	44.4 ± 1.3	7.8 ± 0.3[e]	47.1 ± 1.4	8.2 ± 0.2[e]	31.8 ± 1.4	5.6 ± 0.2[e]
20	38.1 ± 0.8	7.6 ± 0.2[f]	45.8 ± 0.9	9.2 ± 0.2[f]	30.8 ± 1.2	6.2 ± 0.2[f]
25	34.7 ± 0.1	8.7[g]	36.2 ± 0.5	9.2 ± 0.1[g]	25.2 ± 0.2	6.3[g]
30	34.5 ± 1.4	9.6 ± 0.6[h]	32.6 ± 0.2	9.7 ± 0.1[h]	22.3 ± 0.3	6.7 ± 0.4[h]
35	32.3 ± 2.6	11.3 ± 1.1[i]	27.7 ± 0.1	9.7[i]	17.0 ± 1.1	5.9 ± 0.4[i]
40	24.8 ± 0.8	9.9 ± 0.4[j]	12.6 ± 1.9	5.0 ± 0.7[j]	11.4 ± 1.0	4.6 ± 0.4[j]

Each value is mean ± SEM of three identical experiments; mean values with the same row sharing the same alphabetical letter superscripts are statistically significant at $P \leqslant 0.05$.

Table 4
Effect of pH on the stability of water soluble curcuminoids.

pH	Residual amount of water soluble curcuminoids (%)		
	Ste	RebA	SG
6.0	80.3 ± 0.4	81.6 ± 0.9	82.3 ± 2.4
7.0	87.2 ± 4.1	84.2	91.0 ± 0.9
7.5	85.3 ± 2.7	84.8 ± 0.6	93.6 ± 2.8
8.0	99.2 ± 0.4	84.1 ± 3.3	90.9 ± 0.6
10.0	100	99.1 ± 0.6	85.0 ± 0.6

from 69.3 ± 4.0 mg/g to 24.8 ± 0.8 mg/g with Ste, from 70.6 ± 0.6 mg/g to 12.6 ± 1.9 mg/g with RebA, and from 73.1 ± 1.0 mg/g to 11.4 ± 1.0 mg/g with SG. The highest amount of water soluble curcuminoids/ml obtained was 11.3 ± 1.1 mg/ml with 35% (w/v) of turmeric powder with Ste, 9.7 ± 1.1 mg/ml with 30% (w/v) of turmeric powder with RebA, and 6.7 ± 0.4 mg/ml with 30% (w/v) of turmeric powder with SG (Table 3). At the optimum extraction condition for soluble curcuminoids, the relative concentrations of curcumin, DMC, and BDMC in total water soluble curcuminoids were 32.4%, 27.0%, and 30.5%, respectively, with Ste, 31.3%, 27.1%, and 31.4%, respectively, with RebA, and 30.3%, 26.7%, and 32.7%, respectively, with SG by using the AlphaEaseFC 4.0 program.

3.3. Stability of water soluble curcuminoids at different pH

The effect of pH (from 6.0 to 10.0) on the stability of water soluble curcuminoids at different pH values was investigated. As shown in Table 4, water soluble curcuminoids extracted with Ste, RebA, and SG at pH 6.0 exhibited stability of 80.3 ± 0.4, 81.6 ± 0.9, and 82.3 ± 2.4%, respectively. Their stabilities were increased to over 84% when the pH value was increased from 6.0 to 7.0 (Table 4).

3.4. Particle size of water soluble turmeric extract

The size distribution of Ste, RebA, SG, turmeric extracted with ethanol, and water soluble turmeric extracted with Ste, RebA,

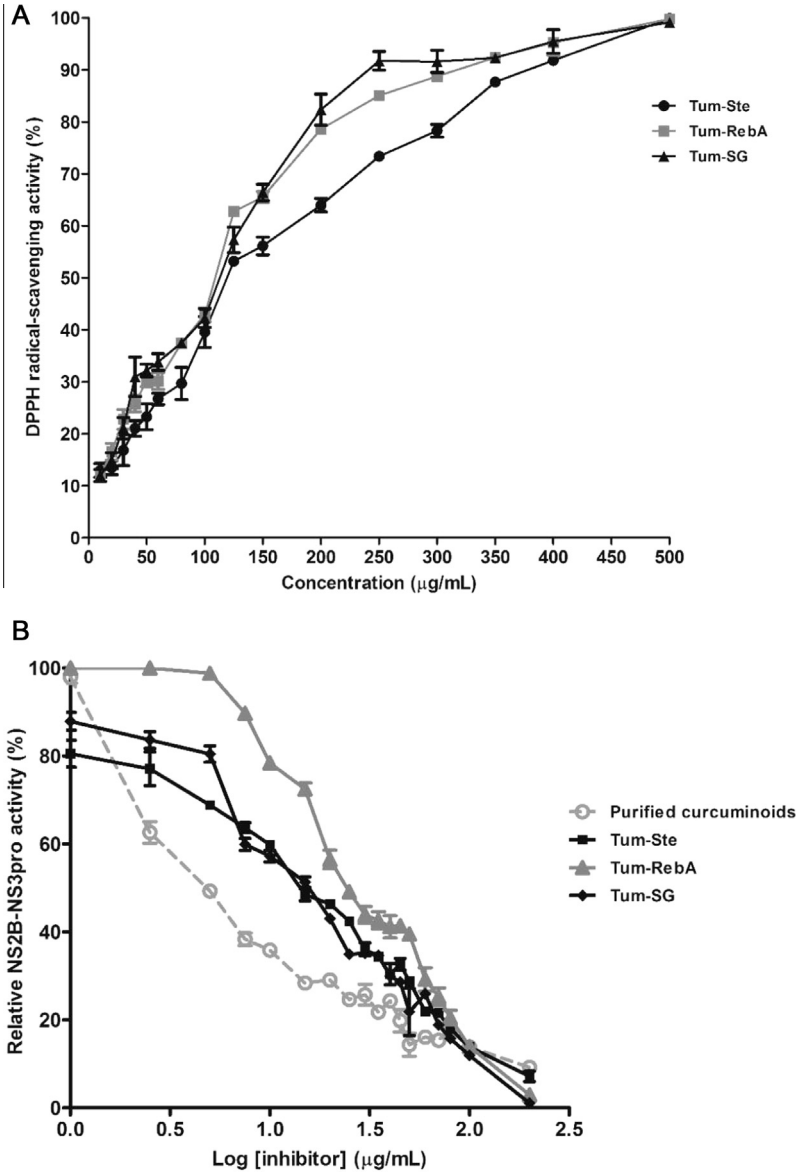

Fig. 2. DPPH radical-scavenging activity (A) and inhibition activity against NS2B-NS3^{pro} of water soluble turmeric extract (B) A: DPPH radical-scavenging activity of turmeric extracts prepared by Ste, RebA, and SG B: The inhibition activity of turmeric extracts against NS2B-NS3^{pro} of dengue fever virus type IV prepared by Ste, RebA, SG, and of purified curcuminoids (⩾94%) Each value is mean ± SEM (n = 3).

and SG are shown in Fig. S1 in the Supplementary materials. The mean particle sizes of Ste, RebA, and SG were 2.4 nm, 3.35 nm, and 2.6 nm, respectively. The particle sizes of turmeric extracted with ethanol and water soluble turmeric extracts prepared with Ste, RebA, and SG were 2.0 nm, 110.8 nm, 95.7 nm, and 32.7 nm, respectively (Table S1).

3.5. Biological function of water soluble turmeric extract

3.5.1. Antioxidant activity

The antioxidant activity of water soluble turmeric extracts using different steviol glucosides was compared using a free radical-scavenging method for DPPH (Fig. 2A). The antioxidant activity was represented by SC_{50} and compared to water soluble turmeric extracted using Ste, RebA, and SG. The SC_{50} values of water soluble turmeric extracts prepared with Ste, RebA, and SG were 127.6 ± 4.9, 105.4 ± 1.8, and 109.8 ± 3.2 μg/ml, respectively. Curcuminoids (⩾94%) containing ⩾80% curcumin and α-tocopherol, and trolox used as positive controls, showed SC_{50} values of 36.2 ± 2.6 μg/ml, 3.5 ± 0.1, and 8.4 ± 0.1 μg/ml, respectively (Fig. S2).

3.5.2. DENV4 NS2B-NS3^{pro} inhibition activity

Water soluble turmeric extracted with Ste, RebA, and SG showed inhibitory activity against NS2B-NS3^{pro} of DENV4 with IC_{50} of 14.1 ± 0.2, 24.0 ± 0.4, and 15.3 ± 0.4 μg/ml, respectively. Detailed inhibition results of water soluble turmeric extracted with Ste, RebA, and SG are shown in Fig. 2B. Curcuminoids (⩾94%) containing ⩾80% curcumin, used as positive controls, showed IC_{50} values of 5.2 μg/ml (Fig. 2B).

4. Discussion

To extract curcuminoids from natural sources, organic solvents, such as acetone, methanol, ethanol, ethyl acetate, isopropanol, and hexane, have been used with soxhlet, ultrasonic, microwave-assisted zone-refining and dipping techniques, and subsequent crystallization (Gopal et al., 2015). However, curcuminoids are hydrophobic polyphenols with poor water solubility. In addition, they are unstable in both *in vivo* and *in vitro* environments with extremely low bioavailability, thus limiting their applications in the food and pharmaceuticals industries (Aditya et al., 2013). To address the poor solubility, poor stability, and poor bioavailability issues of curcumin, several studies have used hydrophobically modified water-soluble polymers that can self-assemble to form micelles in aqueous phase as curcumin platforms (Letchford et al., 2008; Salmaso, Bersani, Semenzato, & Caliceti, 2007). Letchford et al. have shown a 1.3×10^5 fold increase in curcumin solubility in a polymeric micellar formulations containing methoxy poly (ethylene glycol)-*block*-polycaprolactone diblock copolymers (MePEG-*b*-PCL) (Letchford et al., 2008) compared to free curcumin. In addition, the biological half-life of curcumin solubilized in a mixture of DMA, PEG, and dextrose was 60-fold longer than that of free curcumin in rats (Salmaso et al., 2007). Bioconjugation of curcumin, PEG and β-cyclodextrin followed by subsequent functionalization with folic acid (CD-(C6-PEG)5-FA) has shown a 3200 fold increase in solubility of curcumin while decreasing the degradation rate of curcumin at pH 6.5 and 7.2 by 10 and 45-fold, respectively (Salmaso et al., 2007). Until now, most studies have used commercial available curcuminoids extracted by organic solvents. They have enhanced its solubility by using nanoparticles, liposomes, micelles, phospholipid complexes (Ji, Huang, & Zhu, 2012), or rubusoside (Zhang et al., 2011). The processes for preparing water soluble curcuminoids are complicated and expensive. For the first time, we report the simple preparation of water soluble curcuminoids from turmeric powder by using Ste, RebA, or SG. With optimum extraction condition, the yields of soluble curcuminoids by Ste, RebA, and SG were 11.3, 9.7, and 6.7 mg/ml, respectively. The water soluble curcuminoids prepared in this study contained similar amounts of curcumin, DMC, and BDMC. Depending on the targets, curcumin, DMC, and BDC have different activities. BDMC has more activity (cytotoxicity) against ovarian cancer (Syu et al., 1998), for suppressing carcinogenesis (Anto, George, Babu, Rajasekharan, & Kuttan, 1996), reducing nicotine-induced oxidative stress (Kalpana, Sudheer, Rajasekharan, & Menon, 2007), inducing NRF2-mediated induction of heme oxygenase-1 (Devasena, Rajasekaran, Gunasekaran, Viswanathan, & Menon, 2003), and inhibiting human pancreatic α-amylase (Ponnusamy et al., 2012), with more antimutagenic and anticarcinogenic activity (Anto et al., 1996) than curcumin or DMC. Compared to curcumin or BDMC, DMC is more potent in inhibiting the proliferation of breast cancer cells (Simon et al., 1998) or inducing nematocidal activity. Curcuminoids are unstable in solutions of a basic pH and about 90% of curcuminoids were decomposed within 30 min in 0.1 M phosphate buffer (pH 7.2) and serum-free medium (Wang et al., 1997). Curcuminoids are practically insoluble in acidic solutions, and can be broken down easily, producing mainly ferulic acid, feruloylmethane, and yellow brown condensation products (Wang et al., 1997). In aqueous solutions of Ste, RebA, and SG, curcuminoids are stable over a wide range of pHs (2–10) at 80 °C for 2 h or 120 °C for 1 h (Kroyer, 2010). Complexes of water soluble curcuminoids in Ste, RebA, or SG also showed over 80% stability at pH 6.0 and the stability increased to over 84% when the pH value was increased from 6.0 to 10.0 (Table 4). These results were in agreement with the report by Kroyer that Ste has a protective effect resulting from delayed degradation of ascorbic acid (Kroyer, 2010). Water soluble curcuminoids in Ste, RebA, and SG showed nano sizes. As a particle becomes smaller, the surface area to volume ratio increases. The larger surface area allows greater interaction with the solvent, thus increasing solubility (Savjani, Gajjar, & Savjani, 2012). Water soluble curcuminoids have a fairly narrow size distribution because their polydispersity index (PI) is lower than 0.2 (Murdock et al., 2008) (Table S1). Nanoparticle curcuminoids, that is curcuminoids controlled for a distribution between 100 and 1000 nm with mean particle sizes of 190 nm, show improved bioavailability of up to 27-fold in human blood compared to curcumin powder. They also exhibit inhibitory action against intoxication after alcohol consumption in humans by reducing acetaldehyde concentration in the blood (Sasaki et al., 2011). This demonstrates the potential for improving the bioavailability of water soluble curcuminoids through extraction using Ste, RebA, and SG in the future.

Although many studies have reported that the solubility of bioactive molecules can be enhanced using steviol glucosides (Nguyen et al., 2014; Zhang et al., 2011), the enhancing mechanism remains unknown. Cyclodextrins are used as drug carriers to enhance the solubility, stability, and bioavailability of bioactive molecules. They have been approved by the Food and Drug Administration (Davis & Brewster, 2004). Cyclodextrins have a truncated cone shape, with an internal hydrophobic zone and a hydrophilic external surface. Therefore, they are able to form inclusion complexes with poorly water soluble molecules, thus improving the solubility of the molecules (Davis & Brewster, 2004). Ste, RebA, and SG are glycosides on aglycone's carbon skeleton steviol. Ste, RebA, and SG have a D-glucose group affixed at C_{19}. In addition, Ste has a di-glucosyl while RebA has a tri-glucosyl sugar moiety affixed at C_{13}. SG is the glycoside of stevioside. While Ste, RebA, and SG show high solubility in water (Kroyer, 2010), steviol is hydrophobic in the center. According to the yield of water soluble curcuminoids obtained from turmeric powder, as the glucose unit

connected to steviol at C_{13} was increased, the yield of water soluble curcuminoids decreased (Tables 1–3). Analysis of the main products in water soluble curcuminoids showed that DMC had the lowest relative concentration in water soluble curcuminoids. There was a slight decrease in the relative concentration of curcumin when SG instead of Ste was used. But the relative concentration of BDMC was slightly increased when SG instead of Ste was used. Based on these results, the glucose connected to steviol might have enhanced the yield of water soluble curcuminoids and the ratio of each compound. Therefore, a complex with Ste, RebA, and SG might be able to stabilize curcuminoids at different pHs. However, the exact mechanisms of the solubilization of water insoluble compounds require further study.

Water soluble turmeric extracts are a mixture of three main analogues: curcumin, DMC, and BDMC. Their biological activities were evaluated through DPPH radical scavenging activity and inhibition activity against NS2B-NS3^{pro} of DENV4. Somparn et al. reported that among the three main analogues above, curcumin has the strongest DPPH radical scavenging activity. The SC_{50} values of curcumin, DMC, and BDMC are 35.1, 53.4, and more than 200 μg/ml, respectively (Somparn et al., 2007). Water soluble turmeric extract with Ste showed slightly higher DPPH activity than pure BDMC. There were small changes in the ratio of curcumin, DMC, and BDMC in water soluble curcuminoids prepared with Ste, RebA, or SG. In addition, they showed different DPPH activities. Therefore, the ratio of curcumin, DMC, and BDMC in water soluble curcuminoids might affect their DPPH activities.

Chen et al. reported that curcumin can interfere with the infection processes of viruses, such as dengue virus type 2 (Chen et al., 2013). The water soluble turmeric extract prepared with Ste and SG showed higher inhibitory activity against NS2B-NS3^{pro} of DENV4 than water soluble turmeric extract prepared with RebA. Therefore, water soluble curcuminoids extracted with Ste, RebA, or SG maintained their biological activities.

5. Conclusion

Curcuminoids in turmeric *Curcuma longa* has been widely used as pigments in food processing as well as in traditional medicine. For various applications of curcuminoids, water solubility is an important factor to achieve their desired plasma levels for pharmacological response. For the first time, we prepared water soluble curcuminoids directly from turmeric *Curcuma longa* powder by using Ste, RebA, and SG, forming nanoparticles with fairly narrow size distribution. These water soluble curcuminoids have antioxidant activity and inhibition activity against NS2B-NS3pro of dengue virus type 4. This facile method of preparing water soluble curcuminoids come with a significant advantage to be widely applicable for solubilization of various insoluble functional flavonoids in plants, fruits, or food materials.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

This work was partially supported by Agriculture, Food and Rural Affairs Research Center Support Program, Ministry of Agriculture, Food and Rural Affairs, Republic of Korea, by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2015R1D1A1A01056929; D. Kim, and 2015R1D1A4A01020522; T.T. Hanh Nguyen) and by OTTOGI Corporation through Research and Publication Projects.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2016.07.102.

References

Aditya, N. P., Shim, M., Lee, I., Lee, Y., Im, M. H., & Ko, S. (2013). Curcumin and genistein coloaded nanostructured lipid carriers: *In vitro* digestion and antiprostate cancer activity. *Journal of Agriculture and Food Chemistry, 61*, 1878–1883.

Ahsan, H., Parveen, N., Khan, N. U., & Hadi, S. M. (1999). Pro-oxidant, anti-oxidant and cleavage activities on DNA of curcumin and its derivatives demethoxycurcumin and bisdemethoxycurcumin. *Chemico-Biological Interactions, 121*, 161–175.

Anand, P., Thomas, S. G., Kunnumakkara, A. B., Sundaram, C., Harikumar, K. B., Sung, B., ... Aggarwal, B. B. (2008). Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature. *Biochemical Pharmacology, 76*, 1590–1611.

Anto, R. J., George, J., Babu, K. V., Rajasekharan, K. N., & Kuttan, R. (1996). Antimutagenic and anticarcinogenic activity of natural and synthetic curcuminoids. *Mutation Research-Genetic Toxicology, 370*, 127–131.

Chen, T. Y., Chen, D. Y., Wen, H. W., Ou, J. L., Chiou, S. S., Chen, J. M., ... Hsu, W. L. (2013). Inhibition of enveloped vruses infectivity by curcumin. *PLoS ONE, 8*, e62482.

Chen, X., Zou, L. Q., Niu, J., Liu, W., Peng, S. F., & Liu, C. M. (2015). The stability, sustained release and cellular antioxidant activity of curcumin nanoliposomes. *Molecules, 20*, 14293–14311.

Cheng, A. L., Hsu, C. H., Lin, J. K., Hsu, M. M., Ho, Y. F., Shen, T. S., ... Hsieh, C. Y. (2001). Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. *Anticancer Research, 21*, 2895–2900.

Collett, G. P., & Campbell, F. C. (2004). Curcumin induces c-jun N-terminal kinase-dependent apoptosis in HCT116 human colon cancer cells. *Carcinogenesis, 25*, 2183–2189.

Davis, M. E., & Brewster, M. E. (2004). Cyclodextrin-based pharmaceutics: Past, present and future. *Nature Reviews Drug Discovery, 3*, 1023–1035.

Devasena, T., Rajasekaran, K. N., Gunasekaran, G., Viswanathan, P., & Menon, V. P. (2003). Anticarcinogenic effect of bis-1,7-(2-hydroxyphenyl)-hepta-1,6-diene-3,5-dione a curcumin analog on DMH-induced colon cancer model. *Pharmacological Research, 47*, 133–140.

Goel, A., Kunnumakkara, A. B., & Aggarwal, B. B. (2008). Curcumin as "Curecumin": From kitchen to clinic. *Biochemical Pharmacology, 75*, 787–809.

Gopal, J., Muthu, M., & Chun, S. C. (2015). One-step, ultrasonication-mobilized, solvent-free extraction/synthesis of nanocurcumin from turmeric. *RSC Advances, 5*, 48391–48398.

Ireson, C. R., Jones, D. J., Orr, S., Coughtrie, M. W., Boocock, D. J., Williams, M. L., ... Gescher, A. J. (2002). Metabolism of the cancer chemopreventive agent curcumin in human and rat intestine. *Cancer Epidemiology Biomarkers Prevention, 11*, 105–111.

Ji, J. L., Huang, X. F., & Zhu, H. L. (2012). Curcumin and its formulations: Potential anti-cancer agents. *Anticancer Agents Medicinal Chemistry, 12*, 210–218.

Kalpana, C., Sudheer, A. R., Rajasekharan, K. N., & Menon, V. P. (2007). Comparative effects of curcumin and its synthetic analogue on tissue lipid peroxidation and antioxidant status during nicotine-induced toxicity. *Singapore Medicinal Journal, 48*, 124–130.

Kanai, M., Imaizumi, A., Otsuka, Y., Sasaki, H., Hashiguchi, M., Tsujiko, K., ... Chiba, T. (2012). Dose-escalation and pharmacokinetic study of nanoparticle curcumin, a potential anticancer agent with improved bioavailability, in healthy human volunteers. *Cancer Chemotherapy and Pharmacology, 69*, 65–70.

Kroyer, G. (2010). Stevioside and Stevia-sweetener in food: Application, stability and interaction with food ingredients. *Journal of Consumer Protection and Food Safety, 5*, 225–229.

Lao, C. D., Ruffin, M. T. T., Normolle, D., Heath, D. D., Murray, S. I., Bailey, J. M., ... Brenner, D. E. (2006). Dose escalation of a curcuminoid formulation. *BMC Complementary and Alternative Medicine, 6*, 10.

Letchford, K., Liggins, R., & Burt, H. (2008). Solubilization of hydrophobic drugs by methoxy poly(ethylene glycol)-block-polycaprolactone diblock copolymer micelles: Theoretical and experimental data and correlations. *Journal of Pharmaceutical Sciences, 97*, 1179–1190.

Li, L., Braiteh, F. S., & Kurzrock, R. (2005). Liposome-encapsulated curcumin: *In vitro* and *in vivo* effects on proliferation, apoptosis, signaling, and angiogenesis. *Cancer, 104*, 1322–1331.

Lin, X. H., Ji, S., Qiao, X., Hu, H. B., Chen, N., Dong, Y. H., ... Ye, M. (2013). Density functional theory calculations in stereochemical determination of rerpecurcumins J-W, cytotoxic terpene-conjugated curcuminoids from *Curcuma longa* L. *Journal of Organic Chemistry, 78*, 11835–11848.

Liu, A., Lou, H., Zhao, L., & Fan, P. (2006). Validated LC/MS/MS assay for curcumin and tetrahydrocurcumin in rat plasma and application to pharmacokinetic study of phospholipid complex of curcumin. *Journal of Pharmaceutical Biomedical Analysis, 40*, 720–727.

Murdock, R. C., Braydich-Stolle, L., Schrand, A. M., Schlager, J. J., & Hussain, S. M. (2008). Characterization of nanomaterial dispersion in solution prior to *in vitro*

exposure using dynamic light scattering technique. *Toxicological Sciences, 101*, 239–253.
Nguyen, T. T., Jung, S. J., Kang, H. K., Kim, Y. M., Moon, Y. H., Kim, M., & Kim, D. (2014). Production of rubusoside from stevioside by using a thermostable lactase from Thermus thermophilus and solubility enhancement of liquiritin and teniposide. *Enzyme Microbial Technology, 64–65*, 38–43.
Nguyen, T. T. H., Lee, S., Wang, H. K., Chen, H. Y., Wu, Y. T., Lin, S. C., ... Kim, D. (2013). *In vitro* evaluation of novel inhibitors against the NS2B-NS3 protease of dengue fever virus type 4. *Molecules, 18*, 15600–15612.
Nishiyama, T., Mae, T., Kishida, H., Tsukagawa, M., Mimaki, Y., Kuroda, M., ... Kitahara, M. (2005). Curcuminoids and sesquiterpenoids in turmeric (*Curcuma longa* L.) suppress an increase in blood glucose level in type 2 diabetic KK-Ay mice. *Journal of Agriculture and Food Chemistry, 53*, 959–963.
Ohori, H., Yamakoshi, H., Tomizawa, M., Shibuya, M., Kakudo, Y., Takahashi, A., ... Shibata, H. (2006). Synthesis and biological analysis of new curcumin analogues bearing an enhanced potential for the medicinal treatment of cancer. *Molecular Cancer Therapeutics, 5*, 2563–2571.
Ponnusamy, S., Zinjarde, S., Bhargava, S., Rajamohanan, P. R., & Ravikumar, A. (2012). Discovering Bisdemethoxycurcumin from *Curcuma longa* rhizome as a potent small molecule inhibitor of human pancreatic alpha-amylase, a target for type-2 diabetes. *Food Chemistry, 135*, 2638–2642.
Priyadarsini, K. I. (2014). The chemistry of curcumin: From extraction to therapeutic Agent. *Molecules, 19*, 20091–20112.
Salmaso, S., Bersani, S., Semenzato, A., & Caliceti, P. (2007). New cyclodextrin bioconjugates for active tumour targeting. *Journal of Drug Targeting, 15*, 379–390.
Sandur, S. K., Pandey, M. K., Sung, B., Ahn, K. S., Murakami, A., Sethi, G., ... Aggarwal, B. B. (2007). Curcumin, demethoxycurcumin, bisdemethoxycurcumin, tetrahydrocurcumin and turmerones differentially regulate anti-inflammatory and anti-proliferative responses through a ROS-independent mechanism. *Carcinogenesis, 28*, 1765–1773.
Sasaki, H., Sunagawa, Y., Takahashi, K., Imaizumi, A., Fukuda, H., Hashimoto, T., ... Morimoto, T. (2011). Innovative preparation of curcumin for improved oral bioavailability. *Biological and Pharmaceutical Bulletin, 34*, 660–665.
Savjani, K. T., Gajjar, A. K., & Savjani, J. K. (2012). Drug solubility: Importance and enhancement techniques. *ISRN Pharmaceutics, 2012*, 195727.
Shoba, G., Joy, D., Joseph, T., Majeed, M., Rajendran, R., & Srinivas, P. S. (1998). Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. *Planta Medica, 64*, 353–356.
Simon, A., Allais, D. P., Duroux, J. L., Basly, J. P., Durand-Fontanier, S., & Delage, C. (1998). Inhibitory effect of curcuminoids on MCF-7 cell proliferation and structure-activity relationships. *Cancer Letters, 129*, 111–116.
Somparn, P., Phisalaphong, C., Nakornchai, S., Unchern, S., & Morales, N. P. (2007). Comparative antioxidant activities of curcumin and its demethoxy and hydrogenated derivatives. *Biological Pharmaceutical Bulletin, 30*, 74–78.
Syu, W. J., Shen, C. C., Don, M. J., Ou, J. C., Lee, G. H., & Sun, C. M. (1998). Cytotoxicity of curcuminoids and some novel compounds from *Curcuma zedoaria*. *Journal of Natural Products, 61*, 1531–1534.
Upreti, M., Strassburger, K., Chen, Y. L., Wu, S. X., & Prakash, I. (2011). Solubility Enhancement of steviol glycosides and characterization of their inclusion complexes with gamma-cyclodextrin. *International Journal of Molecular Sciences, 12*, 7529–7553.
Wang, Y. J., Pan, M. H., Cheng, A. L., Lin, L. I., Ho, Y. S., Hsieh, C. Y., & Lin, J. K. (1997). Stability of curcumin in buffer solutions and characterization of its degradation products. *Journal of Pharmaceutical and Biomedical Analysis, 15*, 1867–1876.
Zhang, F., Koh, G. Y., Jeansonne, D. P., Hollingsworth, J., Russo, P. S., Vicente, G., ... Liu, Z. (2011). A novel solubility-enhanced curcumin formulation showing stability and maintenance of anticancer activity. *Journal of Pharmaceutical Sciences, 100*, 2778–2789.