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-$NS3^{pro}$ 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