CHAPTER

23

Enzymatic Production of Steviol Glucosides Using β-Glucosidase and Their Applications

Thi Thanh Hanh Nguyen[*], *Changseop Seo*[†], *So-Hyung Kwak*[†], *Jeesoo Kim*[†], *Hee-Kyoung Kang*[‡], *Seong-Bo Kim*[§], *Doman Kim*[*,†]

[*]The Institute of Food Industrialization, Institutes of Green Bio Science and Technology, Seoul National University, Pyeongchang-gun, South Korea [†]Graduate School of International Agricultural Technology, Seoul National University, Pyeongchang-gun, South Korea [‡]Department of Biomedical Science, Chosun University, Gwangju, South Korea [§]CJ CheilJedang, Life Ingredient and Material Research Institute, Suwon, South Korea

23.1 INTRODUCTION

Natural sweeteners have received much interest due to increasing health concerns over the consumption of sugar as well as problems related to the safety of some nonnutritive artificial sweeteners. Steviol glucosides (stevioside, rebaudioside) are extracted from the leaves of the plant *Stevia rebaudiana* Bertoni, a rhizomatous perennial shrub in the family of Asteraceae that originated in Paraguay and Brazil. For the first time, they were approved for consumption as a natural sweetener in the United States, the European Union, Australia, and New Zealand (Risso et al., 2014). The sweet taste of leaves of *S. rebaudiana* is due to the ent-kaurane type diterpenoid glycosides commonly containing aglycone and steviol that differ from each other only in the position (C13 and/or C19) of glycosidic constituent (Ko et al., 2012; Nguyen et al., 2014). More than 30 steviol glycosides at various concentrations are accumulated in *S. rebaudiana* (Wolwer-Rieck, 2012). Glycosides found in *S. rebaudiana* include stevioside (Ste, 5%–10%), rebaudiosides (Reb)A (2%–5%), RebC (1%), dulcoside (0.5%), other Rebs (RebD, RebE, and RebF, ≤0.2%), and rubusoside (≤0.2%) (Table 23.1) (Yadav and Guleria, 2012; Chatsudthipong and Muanprasat, 2009). Steviol glucosides have gained great attention due to their usage as a

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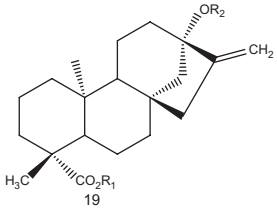
TABLE 23.1 Major Biochemical Components of *Stevia rebaudiana*

Components	Structure		MW	Value (g/100 g dry leaf weight)	Plant Source	Sweetness*	Reference
	R1 (C-19/ Carboxylic Acid)	R2 (C-13/ Hydroxyl)					
Steviol			318.2	Trace <0.01%	*S. rebaudiana*		
	H	H					
Stevioside	β-Glc	β-Glc-β-Glc(2→1)	804.9	5%–10%		300	Yadav and Guleria, 2012; Chatsudthipong and Muanprasat, 2009
RebA	β-Glc	β-Glc-β-Glc(2→1)-β-Glc(3→1)	967.0	2%–5%		250–450	Chatsudthipong and Muanprasat, 2009
RebC	β-Glc	β-Glc-α-Rha(2→1)-β-Glc(3→1)	951.0	1%		50–120	
Dulcoside A	β-Glc	β-Glc-α-Rha(2→1)	788.9	0.5%		50–120	
RebD	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1)-β-Glc(3→1)	1129.2	0.2%		250–450	
RebE	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1)	967.0	0.2%		150–300	
RebF	β-Glc	β-Glc-β-Xyl(2→1)-β-Glc(3→1)	937.0	0.2%			
Steviolbioside	H	β-Glc-β-Glc(2→1)	642.7	0.1%		100–125	
Rubusoside	β-Glc	β-Glc	642.73	Trace		115	Ko et al., 2012
				5.3%	*R. suavissimus* S. Lee		Tanaka et al., 1981

Reb, rebaudioside; Glc, glucosyl; Rha, rhamnosyl; Xyl, xylosyl; Sweetness*, times sweeter than sucrose at concentration of 0.025%.

low-cost natural sweetener in food and beverages. They are intensively sweet, low in calories, nonnutritive, highly stable, and have the therapeutic properties of being antihyperglycemic, antihypertensive, anti-inflammatory, antitumor, antidiarrheal, and antidiuretic while also having immunomodulatory effects (Clos et al., 2008; Wolwer-Rieck et al., 2010; Goyal et al., 2010). Although stevia occupies only 1% of the global artificial sweeteners market, its market is growing at a rate of 4% per annum with an estimated value of around $1.3 billion. Japan solely invests 40% in the international sweetener market. The estimate of the Japanese total market value of stevia sweetener was approximately $25–35 million per year (Megeji et al., 2005). China accounts for 75% of global *Stevia* plant cultivation, which is 80,000 acres of land. The Chinese stevia industry experienced a significant increase in yield, from 2073 tons in 2007 to 3096 tons in 2009, and 80% of the yield was exported. It has been estimated that worldwide stevia sweetener capacity grew from 5000 tons per year in 2007 to 11,789 tons per year in 2009 (Yadav and Guleria, 2012; Chatsudthipong and Muanprasat, 2009). Steviol, steviolbioside, and Ru are rare in nature, unlike Ste. *S. rebaudiana* only contains trace amounts of them. Ru can be extracted from *Rubus suavissimus* S. Lee (Rosaceae). However, the yearly yield of Ru is various depending on regional climates (Wan et al., 2012). Thus, mass purification of Ru was considered to be complicated and economically impractical (Ko et al., 2012; Sugimoto et al., 2002). Hydrolysis of Ste has been suggested as a method because Ste contains three glucosyl groups at the C13 and C19 positions, which can yield steviolbioside, steviol, isosteviol, steviol mono-glucosyl ester, Ru, or steviol mono-glucoside after full or partial cleavage. β-Glucosidase is an enzyme produced by all life domains. It can hydrolyze the β-D-glucosidic bonds of various compounds comprised of alkyl-β-D-glucosides, aryl-β-D-glucosides, cyanogenic glucosides, disaccharides, and short-chain oligosaccharides, liberating glucose from a nonreducing end. Therefore, researchers have tried to hydrolyze Ste by using β-glucosidases to produce specific products for industrial applications with high yields. This review will summarize recent advances in the enzymatic production of steviol glycosides from Ste by using β-glycosidases and the characterization of products with a particular focus on their potential industrial applications. In steviol glucosides, the number of carbohydrate groups at the C13 and C19 sites will determine the degree of sweetness of steviol (Adari et al., 2016; Gerwig et al., 2016). It has been reported that Ste is 300 times sweeter than sucrose (Yadav and Guleria, 2012; Chatsudthipong and Muanprasat, 2009). Although the concentration of Ste in the *S. rebaudiana* leaves is higher than that of RebA, the bitter aftertaste of Ste and its low solubility in water limit its use for human consumption. That also limits its application in food and pharmaceutical products (Adari et al., 2016; Ko et al., 2016). Compared to Ste, RebA has an extra glucosyl group at the C13 site. It imparts the greatest potency of sweetness with a less bitter aftertaste (Kohda et al., 1976; Adari et al., 2016).

23.2 ENZYMATIC MODIFICATION OF STEVIOL GLUCOSIDES

23.2.1 Production of Steviol

Ste contains three β-glycosidic bonds (β-linked sophomore, β-1,2-D-glucopyranosyl on C13, and an ester β-glucosidic linkage on the C19 carboxyl group). Ru can be produced by selective cleavage of β-1,2-glucosidic linkage of the sophorosyl moiety at site C13, whereas hydrolysis of different positioning glycosides with different numbers of Ste will produce

steviol, isosteviol, steviolmonoside, steviolbioside, and a mixture of these compounds (Okamoto et al., 2000). Unlike Ste, steviol is rare in nature. Therefore, few studies have reported its synthetic methods (Ogawa et al., 1980; Ko et al., 2013; Nguyen et al., 2016; Milagre et al., 2009; Chen et al., 2014). Steviol is an aglycone of steviol glucosides. It has been pharmaceutically used to improve cognitive functions including memory, alertness, learning, and psychotic stability. In addition, it has been used as a plant-growth factor (Ko et al., 2013; de Oliveira et al., 2008). It might also have potential antihyperglycemic effects on stimulating pancreatic beta cells to secrete insulin (Jeppesen et al., 2000). Wang and Lu have separated 4 μg of steviol/g dry weight of *R. suavissimus* (Wang and Lu, 2007) or 5.9 ± 0.8 μg of steviol/g dry weight of *S. rebaudiana* by high-performance liquid chromatography (Minne et al., 2004). To produce steviol, a chemical method involving the hydrolysis of Ste under extremely acidic conditions has been used. However, steviol produced in that way is rearranged into isosteviol automatically (Kohda et al., 1976). Briefly, to produce steviol, 1 g Ste and 1.5 g sodium periodate in 75 mL water are stirred for 16 h. Then 7.5 g potassium hydroxide is added and refluxed for 1 h. The mixture is then carefully acidified with acetic acid and extracted using ether. The organic layer is then washed with water, dried using magnesium sulfate, and concentrated in vacuo to give crystalline residue (Ogawa et al., 1980). Extraction and crystallization can afford steviol with a yield of 75%. This process requires a highly diluted system and a large amount of costly sodium periodate to achieve meaningful yields (Ogawa et al., 1980; Ko et al., 2013). To produce steviol from Ste, enzymatic methods have also been reported (Wan et al., 2012; Ko et al., 2013; Milagre et al., 2009; Nguyen et al., 2016; Chen et al., 2014; Mizukami et al., 1982) (Table 23.2). Steviol yield has been reported to be 20% by using pancreatin with ethanol as a cosolvent at pH 7.0 (Milagre et al., 2009). The yield is 10% with fungal lipase/ethanol at pH 4.0 and 20.8% with *Aspergillus niger* at pH 7.0 for seven days (Milagre et al., 2009). Crude hesperidinase containing β-1,4-rhamnoglucosidase and flavonoid-β-glucosidase from a culture medium of *A. niger* has been used to break the glycoside bonds of Ste into steviol (Mizukami et al., 1982). Among nine screened commercial enzymes produced from *A. niger* (hemicellulose, hesperidinase, and β-glucanase), *Tricoderma longibrachiatum* (β-glucanase), *Aspergillus aculeatus* (Viscozyme L), *Trichoderma reesei* ATCC 26921 (β-glucanase), *Clostridium thermocellusm* (thermostable β-glucanase), or *Penicillium decumbens* (naringinase), and almond β-glucosidase, two enzymes prepared from *A. aculeatus* and *P. decumbens* have been found to be able to hydrolyze the glucosidic linkage of sophoroside at the 13-hydroxyl group or glucose at the 19-carboxyl group, thus producing the following three products from Ste: Ru, steviol monoside, and steviol (Ko et al., 2013) (Table 23.2). Naringinase (EC 3.2.1.40) has an activity of α-rhamnosidase responsible for naringin hydrolysis to produce prunin (4,5,7-trihydroxy flavovone-7-glucoside) and rhamnose. It also contains an activity of β-glucosidase that can hydrolyze prunin into naringenin (4′,5,7-trihydroxyflavanone) and glucose (Ribeiro and Ribeiro, 2008). The naringinase from *P. decumbens* has both activities of α-L-rhamnosidase and β-D-glucosidase for the hydrolysis of naringin to produce naringenin as the final product (Lee et al., 2013). This enzyme exhibits higher hydrolyzing activity against Ste, Ru, steviol mono-glucoside, and steviol mono-glucosyl ester than β-glucobioses. It seldom hydrolyzes RebA containing a β-glucosyl (1–3) unit at the C-13-hydroxyl group of Ste (Ko et al., 2013). The major pathway for steviol synthesis by β-glucosidase from *P. decumbens*is is Ste to Ru to steviol mono-glucoside to steviol. Steviol yield by *P. decumbens* β-glucosidase has been reported to be 64% using 47 mM Ste

TABLE 23.2 Sources of Glycoside Hydrolases Used for Bioconversion of Steviol Glucosides

Sources	Enzyme	pH	*T* (°C)	Substrate	Products	Yields (%)	Reference
Penicillium decumbens	β-Glucosidase	4.0	55	Stevioside	Steviol	64	Ko et al., 2013
Sulfolobus solfataricus	β-Galactosidase (mutant)	4.5	80	Stevioside	Steviol	97.4	Chen et al., 2014
	β-Galactosidase	6.0	75	Stevioside	Steviol	99.2	Nguyen et al., 2016
Aspergillus sp.	β-Galactosidase	4.5	60	Stevioside	Rubusoside	91.4	Wan et al., 2012
Aspergillus aculeatus	β-Glucosidase	5.1	63	Stevioside	Rubusoside	66	Ko et al., 2012
Thermus thermophilus	β-Glucosidase	7.0	70	Stevioside	Rubusoside	92	Nguyen et al., 2014
Streptomycess sp. GXT6	β-Glucosidase	8.5	50	Stevioside	Rubusoside	78.8	Wang et al., 2015
Clavibacter michiganense	β-Glucosidase	4.5	40	Stevioside	Steviolbioside, rubusoside	ND	Nakano et al., 1998
				Rubusoside	Steviolmonoside		
				Rebaudioside A	Rebaudioside B		
				Steviol-19-*O*-glucoside	Steviol		
Flavobacterium johnsonae	β-Glucosidase	7.0	40	Stevioside, rebaudioside	Steviolbioside, rebaudioside B	ND	Okamoto et al., 2000
				Rubusoside	Steviolmonoside, steviol-19-*O*-glucoside		

at 55°C with pH 4.0 (Ko et al., 2013). Recently, β-glycosidase from *Sulfolobus solfataricus* has been used to hydrolyze Ste to steviol with high yield (99.2%) at 75°C for 12 h (Nguyen et al., 2016; Chen et al., 2014) (Table 23.2). This β-glucosidase can hydrolyze β-glycosidic bonds in Ru, Ste, and RebA to produce steviol with different efficiencies (Nguyen et al., 2016). Steviol yields from Ste, Ru, and Reb have been reported to be 98%, 44.2%, and a negligible amount, respectively (Nguyen et al., 2016). Reaction kinetic studies of *S. solfataricus* β-glycosidase (SSbgly) using Ste as substrate have revealed that K_m and k_{cat} values are 17.21 mM and 1.62 s^{-1}, respectively. The k_{cat}/K_m ratio of SSbgly for Ste is 0.094 $s^{-1}mM^{-1}$ (Nguyen et al., 2016). β-Glycosidase from *S. solfataricus*, ahyperthermophilic bacterium grown in volcanic springs with optimal growth at 75–80°C, is one of the most thermostable glycosyl hydrolases (Wu et al., 2013). Currently, among the reported methods, β-glycosidase from *S. solfataricus* has shown the strongest conversion of Ste (>99% of used Ste) to steviol with a yield of over 98%. This simplified purification method for SSbgly by heat treatment can result in activity recovery of >95% (Nguyen et al., 2016). Thus, it is applicable as an efficient and cost-effective steviol production method with the potential for large-scale industrial application.

23.2.2 Production of Rubusoside

Rubusoside (13-*O*-β-glucosyl-19-*O*-β-D-glucosyl-steviol, Ru) is the major component (5.3 mg/g dry leaves (Tanaka et al., 1981) in leaves of *R. suavissimus* S. Lee (Rosaceae), commonly called tiancha in Chinese or Chinese sweet tea, that is cultivated throughout southwestern China. Trace amount of Ru has also been found in *S. rebaudiana* Bertoni (Chaturvedula and Prakash, 2011; Koh et al., 2009). Ru is a good candidate for a natural sweetener due to its sweetness, which is approximately 115 times sweeter than sucrose at a concentration of 0.025% (Koh et al., 2009). On top of its role as a sweetener, Ru also has been used as a solubility enhancer of pharmaceutical compounds to improve their bioavailability (Nguyen et al., 2014, Zhang et al., 2012, 2011a). Because growth of *R. suavissimus* S. Lee has variable annual yields depending on regional climates (Wan et al., 2012), mass purification of Ru is complicated and economically impractical (Ko et al., 2012; Sugimoto et al., 2002). Indeed, Ru costs at least 10 times more than Ste (Ko et al., 2012). Thus, researchers have tried to produce Ru by selectively cleaving the β-1-2-glucosidic linkage of sophorosyl moiety at site C13 of Ste. Wan et al. (Wan et al., 2012) have reported that β-galactosidase from *Aspergillus* sp. can hydrolyze a glucosyl unit of Ste to produce Ru with a yield of 91.4% at 60°C for 72 h, resulting in Ru synthesis of 0.19 g/L/1000 U/h (Table 23.2). Because this enzyme is specific for hydrolyzing Ste, none of its analogs, such as RebA, RebC, and other steviol glycosides in commercial crude *S. rebaudiana* leaf extract, can be hydrolyzed by this enzyme (Wan et al., 2012). Among nine commercial glycosidases from *A. niger*, *T. longibrachiatum*, *A. aculeatus*, *T. reesei*, *Clostridium thermocellum,* and almonds, only β-glucosidase from *A. aculeatus* (Viscozyme L) can hydrolyze the glucosidic linkage at the sophoroside of Ste. In comparison with other β-glycosidases, this β-glucosidase exhibits higher specificities toward Ste and Ru. However, it can rarely hydrolyze the β-1-3-glucosidic linkage at the 19-carboxyl moiety of RebA or steviol mono-glucosyl ester (Ko et al., 2012). By using β-glucosidase from *A. aculeatus*, an Ru yield of 66% has been obtained from a 280 mM Ste at 63°C with pH 5.1 (Ko et al., 2012) (Table 23.2). Nguyen et al. have screened 31 commercial enzymes with mixed activities of pectinase, hemicellulases, α-galactosidases, cellulases, β-galactosidase, and purified recombinant β-galactosidase from *Thermus thermophilus* expressed in *E. coli* for the transformation of Ste to produce Ru (Nguyen et al., 2014; Lim et al., 2016) (Table 23.2). Among them, commercial Sumizyme SPC from *A. niger*, Sumilact L from *A. niger*, Validase AGS from *A. niger*, naringinase from *Penicillium* sp., and recombinant lactase from *T. thermophiles* have been found to be able to convert Ste to Ru with efficiencies of about 59%, 35%, 56%, 51%, and 92%, respectively (Nguyen et al., 2014). By comparing the amount of Ru synthesis per 1000 U/h among reported papers (Wan et al., 2012), lactase from *T. thermophiles* has shown 23.2 and 56.8 times higher yields than the reported yield of Ru (0.19 g/L/1000 U/h) using β-galactosidase from *Aspergillus* sp. (Wan et al., 2012; Nguyen et al., 2014). Lactase from *T. thermophilus* is a thermostable enzyme. Compared to mesophilic enzymes, thermostable enzymes exhibit maximum activity at a temperature range from 70 to 90°C with significant benefits such as increased reaction velocity, decreased contamination of microorganisms, and extended half-lives of enzymes under reaction conditions (Petzelbauer et al., 1999; Maciunska et al., 2000). After heating the solution containing crude β-galactosidase, 78% of mesophilic proteins are removed,

with the recovery of β-galactosidase activity at 89% (Lim et al., 2016), higher than the recovery yield for partial and purified β-glucosidase from *A. aculeatus* (at 44.7% and 1.8%, respectively) (Ko et al., 2012). To reduce costs, reuse the enzyme, continuously process, and reduce autodigestion in large-scale production, alginate beads have been prepared for an immobilized lactase reactor (Nguyen et al., 2014) or double jacket immobilized lactase columns (Lim et al., 2016). The Ru yield using immobilized lactase has been found to be 1.2 times higher than that using a free enzyme (Nguyen et al., 2014). Wang et al. reported that β-glucosidase obtained by *Streptomyces* sp. GXT6 can convert 98.2% of Ste to 78.8% Ru at pH 8.5 and 50°C for 6 h (Wang et al., 2015) (Table 23.2). This enzyme can also hydrolyze sorphorose, laminaribiose, cellobiose, amygdalin, gentiobiose, esculin, and salin. Enzyme kinetic parameters of this β-glucosidase for Ste are as follows: K_m 1.47 mM, $V_{max} = 16.83\ \mu mol/min/mg$, $k_{cat} = 13.18\ s^{-1}$, and $k_{cat}/K_m = 8.97\ s^{-1}\ mM^{-1}$. To produce Ru, β-glucosidase can specifically hydrolyze the glucosyl moiety of sophoroside at position C13 in Ste. Therefore, it is a prospective candidate for the commercial production of Ru.

23.2.3 Production of Steviolbioside

Steviolbioside 13-[(2-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxyl]kaur-16-en-18-oic acid] is a natural sweetener found in *S. rebaudiana* leaves in rare amounts (Ibrahim et al., 2014). Synthetic methods for steviolbioside such as alkaline and enzymatic hydrolysis of Ste have been investigated (Ko et al., 2013; Chen et al., 2016). Steviolbioside is a byproduct in the production of steviol from Ste using purified β-glucosidase obtained by *P. decumbens* naringinase (Ko et al., 2013). Chen et al. have screened the following six commercial or homemade glycosidases and lipase: β-galactosidase from *Kluyveromyces lactis* (Maxilact LG 2000), β-galactosidase from *S. solfataricus*, β-glucosidase from *A. niger* (Novozymes), β-glucosidase from *Penicillium multicolor* (Aromase), *Candida antarctica* (Novozyme 435), and *Rhizomucor miehei* (Lipozyme RM IM) (Chen et al., 2016). Among them, β-galactosidases from *K. lactis* and *A. niger* have shown high specific activity for the glycosyl ester linkage hydrolysis of Ste, producing steviolbioside as the primary product. A steviolbioside yield of 96% has been obtained at 25 mg Ste/mL, 40°C, pH 7.0, and 9000 U/g Ste for 12 h (Chen et al., 2016).

23.2.4 Other Bioconversion Products From Stevioside

β-Glucosidases from *Clavibacter michiganese* and *Flavobacterium johnsoniae* can hydrolyze glucosyl ester linkages at C19 position of RebA, Ste, Ru, or steviol mono-glucosyl ester. They can also hydrolyze the glucosidic bond in the saccharide at C-13 site of RebB and steviolbioside to polymerize steviol glycosides at a lesser degree (Nakano et al., 1998; Okamoto et al., 2000). However, β-glucosidase from *C. michiganese* cannot cleave the glucosyl residue of RebA, Ste, Ru, or steviol mono-glucoside at the C-13 position, resulting in steviol mono-glucoside as the product after its hydrolysis of steviol glucoside (Nakano et al., 1998) (Table 23.2). β-Glucosidase from *F. johnsoniae* can hydrolyze rubusoside to steviol monoside and seviol-19-glucoside, producing steviol as the final product (Okamoto et al., 2000) (Table 23.2).

23.3 APPLICATIONS OF STEVIOL GLUCOSIDES

23.3.1 Natural Solubilizer

Bioavailability is the major challenge facing the design of oral administration for any drug. Several factors affect oral bioavailability, including water solubility, permeability of the drug into cells, the rate of dissolution, presystemic metabolism, and sensitivity to the efflux mechanism (Savjani et al., 2012). Among those factors, poor solubility and permeability are the most frequent causes of low oral bioavailability (Savjani et al., 2012). Poor water solubility was observed in 70% of new pharmaceutically active ingredient candidates in recent years (Kawabata et al., 2011). Drugs, which are poorly soluble in water, are absorbed slowly inside the body; this leads to inadequate bioavailability with both stomach and intestine mucosal toxicity, thus postponing clinical development of the drug (Le Garrec et al., 2004; Savjani et al., 2012). Some steviol glycosides including Ste, Reb A, and Ru have been found to have solubilizing properties (Table 23.3). Zhang et al. reported that 10% Ru can enhance the solubility from 0.8×10^{-2} mg/mL at 0.1% Ru to 8.5 mg/mL at 10% Ru for etoposide (Zhang et al., 2012) and from 0 to 2.32 mg/mL at 10% Ru, respectively (Zhang et al., 2011a,b). Sizes of Ru-etoposide complex and Ru-curcumin complex are 6.3 ± 0.6 nm and 8 nm, respectively. These water-soluble complexes can maintain their anticancer activities (Zhang et al., 2012). They published that paclitaxel solubility in water can be increased from 1.6 to 6.3 mg/mL with 10%–40% Ru at a particle size of 6.6 nm. In addition, over 80% of this complex has remained soluble in the gastric and intestinal fluids (Liu et al., 2015). Moreover, compared to taxol, the complex of paclitaxel-Ru has almost four times greater permeability in the Caco-2 cell monoculture (Liu et al., 2015) (Table 23.3). Nguyen et al. have reported that Ru can enhance the liquiritin solubility in water from 0.98 to 4.7 mg/mL (Nguyen et al., 2014). Furthermore, it can increase the solubility of teniposide (from 0 to 3.42 mg/mL) (Nguyen et al., 2014) and quercetin (from 0 to 7.7 mg/mL) (Nguyen et al., 2015). The Quercetin-Ru complex can also improve the inhibition activity of quercetin against 3CLpro of severe acute respiratory syndrome (SARS) and human intestinal maltase while maintaining its DPPH radical scavenging and mushroom tyrosinase-inhibiting activities (Nguyen et al., 2015). RebA can enhance the water solubility of quercetin from 0 to 1.46 mg/mL (Nguyen et al., 2015) (Table 23.3). Curcuminoids from turmeric powder, *Curcuma longa* L., have scavenging activities against reactive oxygen species (ROS) and free radicals (Ahsan et al., 1999), effective for antidiabetic usage by decreasing blood glucose levels (Nishiyama et al., 2005), and nematicidal activities (Kiuchi et al., 1993). They can also suppress the proliferation of various tumor cells of the head, neck, lung, pancreas, breast, and prostate as well as against leukemia (Sandur et al., 2007). Although curcuminoids are highly safe and well tolerated by patients, even at very high doses ($\leq$12 g per day) without showing toxicity in vivo studies (Shoba et al., 1998; Cheng et al., 2001; Lao et al., 2006), they have not yet been approved as a therapeutic component due to their poor water solubility (Araujo et al., 2010), chemical unstableness in alkaline solutions (Wang et al., 1997; Price and Buescher, 1997), rapid metabolism (Pan et al., 1999), poor membrane permeation (Wahlang et al., 2011), low bioavailability, and insufficiency to reach the blood concentrations required to affect disease markers or clinical end points, even at chronic doses up to 12 g per day (Lao et al., 2006). The Joint FAO/WHO Expert Committee on Food Additives only approved the use of curcuminoids as food additives if they are extracted

TABLE 23.3 Solubilization of Insoluble Compounds Using Steviol Glucosides and Their Properties

Steviol Glucosides	Compound	Solubility (mg/mL)	Size (nm)	Biological Activity of Soluble Complex	Reference
Rubusoside (10%, w/v)	Etoposide	8.46	6.3 ± 0.6	- Reduced the viability of HT-29, MDA-MB-231, and PC3 cancer cells	Zhang et al., 2011a,b
	Liquiritin	4.7	ND	ND	Nguyen et al., 2014
	Teniposide	3.42			
	Curcumin	2.32	8.0	- Reduced the viability of Caco-2, HT-29, MDA-MB-231, and PANC-1 cancer cells	Zhang et al., 2011a,b
	Quercetin	7.7	ND	- Enhanced inhibition activity against human intestinal maltase, 3CL-protease of SARS - Maintained mushroom tyrosinase inhibition activity	Nguyen et al., 2015
Rebaudioside A (10%, w/w)		1.5		ND	
Rubusoside (40%, w/v)	Paclitaxel	6.26	6.6	- Resulted 4.0 times increase in permeability in Caco-2 cells monocultures - Maintained anticancer activity similar with taxol in DMSO	Liu et al., 2015
Stevioside (8%, w/v)	Extracted curcuminoids from turmeric, *Curcuma longa*	11.3	110.8	- Maintained antioxidant activity and inhibition activity against NS2B-NS3pro of dengue virus type 4	Nguyen et al., 2017
Rebaudioside A (8%, w/v)		9.7	95.7		
Stevioside glucosides (8%, w/v)		6.7	32.7		

from natural source materials. From turmeric powder, Nguyen et al. have directly obtained 11.3, 9.7, and 6.7 mg/mL of water-soluble curcuminoids by using Ste, RebA, and stevioside glucosides (SG), respectively (Nguyen et al., 2017). These water-soluble extracts are nano-sized particles with >80% stability at pH 6.0–10 solutions (Nguyen et al., 2017). The prepared water-soluble turmeric extracts with Ste, RebA, and SG showed inhibition activities (IC_{50}) against NS2B-$NS3^{pro}$ of dengue virus type IV with the value of 14.1, 24.0, and 15.3 μg/mL, respectively (Nguyen et al., 2017) (Table 23.3). Wheat bran (WB) is obtained during the milling process as a by-product, and it contains abundant nutrients and bioactive substances such as carbohydrates (60%), protein (12%), fat (0.5%), minerals (2%), phenolic acids, arabinoxylans,

flavonoids, caroteinoids alkylresorcinol, and phytosterols (Onipe et al., 2015; Slavin, 2003; Javed et al., 2012), known to have health-promoting properties by controlling the glycemic index, decreasing the cholesterol level in plasma, and suppressing the growth of human colon cancer cells. Furthermore, they possess potential prevention properties against oxidative, microbial, inflammatory, and carcinogenic activities (Pruckler et al., 2014; Jensen et al., 2006; Liu et al., 2012; Brouns et al., 2012). Lim et al. have extracted water-soluble polyphenol from WB by using Ru, Ste, RebA, and steviol glucosides (Lim et al., 2016). Total phenol contents in a WB extract prepared by Ru, Ste, RebA, and SG have been found to be 1.19, 1.13, 1.23, and 1.13 times higher, respectively, than that of WB extract prepared using water (Lim et al., 2016). DPPH radical scavenging activities (SC_{50}) of water-soluble WB extracts prepared using water, Ru, Ste, RebA, and SG are 8.76 ± 0.3, 4.87 ± 0.3, 5.34 ± 0.22, 7.27 ± 0.1, and 7.82 ± 0.02 mg/mL, respectively. Thus, WB extracts prepared by Ru, Ste, RebA, and SG possess higher antioxidant activities than WB extracts prepared using water (Lim et al., 2016).

23.3.2 Fructose Transporter (GLUT5) Inhibitor

Among various glucose transporters in human cells, GLUT1 is expressed in most tissues (Yoshikawa et al., 2011; Lange and Brandt, 1990). The over-expressed GLUT1 might be relevant to obesity and noninsulin-dependent diabetes (Miele et al., 1997). Unlike GLUT1, GLUT5 is generally expressed in the small intestine and absorbs fructose from the lumen (Ellwood et al., 1993). Overconsumption of fructose is considered to cause deleterious metabolic effects, thus GLUT5 becomes an increasingly important target for human health (Basciano et al., 2005; Rutledge and Adeli, 2007). Unlike glucose, insulin does not regulate fructose in serum (Litherland et al., 2004). An increase in fructose consumption is correlated with production of lipogenesis and triglyceride at an organism level, and it leads to insulin resistance (Basciano et al., 2005, Rutledge and Adeli, 2007). George Thompson et al. have studied the inhibition effect of Ru on the GLUT1 and GLUT5 of humans expressed in insect cell culture and found that Ru can inhibit both GLUT1 and GLUT5 with IC_{50} values of 4.6 and 6.7 mM, respectively, while Ste does not have such inhibition activities (George Thompson et al., 2015). By using the in silico docking method, Rub was found to interact to the active sites of GLUT1 and GLUT5 in distinguishable form due to a key residue of tryptophan in GLUT1 and alanine in GLUT5 (George Thompson et al., 2015). Ru is a natural solubilizer that can combine with other GLUT drugs to enhance their solubility. In addition, it has effects against GLUT1 and GLUT5.

23.3.3 Effect on Renal Function

By interfering with basolateral entry, Ste and steviol can inhibit transepithelial transport of *para*-aminohippurate (PHA) in rabbit renal proximal tubules (Srimaroeng et al., 2005). Ste has no inhibition effect on either PAH (human organic anion transporter 1, hOAT1) or ES (estrone sulfate, hOAT3) absorbance. However, steviol can significantly and amount-dependently inhibit PAH and ES uptake in hOAT1 and hOAT3 (Srimaroeng et al., 2005). IC_{50} of steivol for hOAT1-mediated PAH transport has been reported to be 11.1 μM compared to 62.6 μM in the case of hOAT3-mediated ES absorbance (Srimaroeng et al., 2005). Michaelis-Menten inhibition constants (K_i) for steviol transport mediated by hOAT1 and hOAT3 are reported to be

2.0 ± 0.3 and 5.4 ± 2.0 μM, respectively (Srimaroeng et al., 2005). As low as 1 μM steviol can increase the efflux of [^{3}H]PAH (transstimulated) through both hOAT1 and hOAT3 (Srimaroeng et al., 2005). Therefore, steviol has great promise to minimize the renal removal of anionic drugs and their metabolites (Srimaroeng et al., 2005).

23.4 CONCLUSION

Steviol glucosides have various applications including their use as an alternative sugar for food, as an ingredient for pharmaceuticals, and as solubilizing agents. Traditionally, steviol, rubusoside, steviolmonoside, steviolbioside, and rebaudioside B are in trace amounts in the stevia leaf, limiting their availability. With our method, the soluble complex of steviol glucosides with less-soluble compounds increased its permeability while retaining the biological activity. Therefore, the production of compounds from stevioside by using the immobilized β-glucosidase with high conversion yields has great potential to be used in industrial applications.

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