Steviol Glycosides from *Stevia rebaudiana* Bertoni: Functional Properties, Safety and Application in Food Industry

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**Abstract** *Stevia rebaudiana* Bertoni, an ancient Paraguayan shrub of the Asteraceae family, is the main source of steviol glycosides (SGs). SGs are a group of intensely sweet diterpene glycosides that are zero-calorie sweeteners, approximately 300 times sweeter than sucrose. Besides having sweetness properties, they are thought to possess some biological activities such as anti-diabetic, anti-diarrheal, anti-tumor and anti-hypertensive activities. Recently, SGs are an important part of the food and beverage industry since consumer demand for natural sweeteners is increasing. This review presents an overview on safety, health benefits and current applications of SGs.

**Key words:** *Stevia rebaudiana*, steviol glycosides, application, safety, health

**Introduction**

In recent years, the rate of metabolic disorders associated with high sugar consumption, such as obesity and type-II diabetes, has been increasing dramatically. Due to this problem, sugar-free and reduced calorie foods and beverages are becoming very popular all around the world. In order to provide alternatives for sucrose (saccharose), many artificial sweeteners have been developed by researchers and companies. Despite their benefits, artificial sweeteners are reported to cause some health risks in various studies [1-3]. These problems have created an increasing demand for the development of natural sweeteners. One of the most promising alternative sugar compounds is a family, called steviol glycosides (SGs). SGs are highly sweet diterpene glycosides obtained from the leaves of *Stevia rebaudiana* Bertoni from Asteraceae family. On average, they are 300 times sweeter than sucrose [4]. There are over 30 SGs identified in *S. rebaudiana* Bertoni leaves. The main sweeting components are stevioside and rebaudioside A [5]. Besides having sweetness properties, SGs are thought to possess health benefits such as, anti-diabetic, anti-tumor, anti-diarrheal and anti-hypertensive activities [6-9].

In a few countries, *Stevia* has been consumed as a food and medicine for many years, including most notably Japan and Paraguay. In 2000s, many national and international food safety authorities have granted the use of *Stevia* extract and high concentration SGs in food and beverages. The application of *Stevia* and its glycosides in the food industry has been rising since then. This paper summarizes the information about properties of SGs, potential health benefits and biological activities, safety and applicability in food and beverage industries.

*Stevia rebaudiana* Bertoni

*Stevia* is a genus from the Asteraceae family, containing approximately 220-230 herbs and shrubs [4]. In 1797, the name “*Stevia*” was given by Antonio José Cavanilles, in memory of a botany professor Petri Iacobi Stevii [10]. Amongst the 230 species in the genus, *Stevia rebaudiana* and *Stevia phlebophylla* are the only members that produce sweet diterpene glycosides, SGs [11].

*Stevia rebaudiana* Bertoni is a branched perennial shrub, previously named as *Eupatorium rebaudianum* and botanically classified by Moises Santiago Bertoni in 1899. Again by M. S. Bertoni, the name of the plant was changed to *Stevia rebaudiana* Bertoni in 1905 [12]. Today, “*Stevia*” is the generic word to describe *S. rebaudiana* Bertoni. *Stevia* is
native to Paraguay, called as “sweet herb of Paraguay”. The plant also occurs in the neighbouring parts of Brazil and Argentina. Other names for Stevia are honey leaf, sweet herb, sweet leaf, candy leaf and honey yerba. With modern cultivation technics, Stevia is grown in many countries across four continents as of today [13]. Stevia is a short day plant that grows up to 1 m tall. It has sessile, elliptic, 3-4 cm long leaves. The root system of the plant is extensive; the stem is woody and weak-pubescent at the bottom. Flowers are white with a pale purple throat, arranged in the small corymbs form [14-15]. Stevia naturally grows at semi-humid subtropical areas, approximately 200-400 meters above the sea level. Average rainfall requirement is 1500-1800 mm and temperature conditions can be variable [13]. Despite the occasional toleration of temperatures near to zero, Stevia suffers from the cold. 20-24 °C are favorable for rapid growth [15]. Moreover, Stevia has a remarkable water need, should be watered frequently. Generally, this period is weekly repeated. In case of lack of water, leaves and stem can wilt quickly, but also recover quickly if the stress conditions no longer exist. The plant can be grown in poor soil, but soils with high salinity should be avoided [16]. Since ancient times Stevia has been used for various purposes such as a sweetener and a medicine [17]. But it is generally known for its sweet molecules, diterpene glycosides.

**Steviol Glycosides**

Glycosides are molecules in which a sugar is bound to a functional group via glycosidic bond. Active and inactive forms of glycosides are abundant in plants. They play many important roles in organisms. These molecules can be converted into a sugar and a non-sugar (aglycone) components by hydrolytic cleavage [18]. Sweet diterpene glycosides from Stevia rebaudiana leaves are known as steviol glycosides (SGs) because they are derivates of steviol molecule. All diterpene glycosides isolated from S. rebaudiana leaves have the same steviol backbone. They differ mainly in the content of carbohydrate residues (R1 and R2), mono-, di-, and trisaccharides containing glucose and/or rhamnose at positions C13 and C19 [19]. SGs are tetracyclic diterpenoids. For 45 years after its first discovery, stevioside was considered as the only SG present in S. rebaudiana. But in 1975, researchers from University of Hiroshima managed to obtain rebaudioside A and B from methanolic leaf extract of the plant [20-21]. Subsequently, seven more SGs from S. rebaudiana had been identified, namely, steviolbioside, rebaudioside C, D, E and F, dulcoside A. In recent years, scientists have identified more SGs, present in trace quantities. Today, over 30 SGs have been detected in S. rebaudiana leaves. Their total contents range from 4 to 20% fresh leaf weight, depending on several conditions, such as cultivation and tillage techniques. Concentration of each compound also shows a differs, but the most abundant SGs (Table 1) and their percentages calculated on a dry weight basis in Stevia leaves are; stevioside (5-10% w/w), rebaudioside A (2-5%), rebaudioside C (1%), dulcoside A (0.5%), rebaudioside D (0.2%), rebaudioside E (0.2%), rebaudioside F (0.2%) and steviolbioside (0.1%) [5]. Additionally, levels of SGs vary in plant organs. The glycosides chiefly present in the leaves. Flowers, stems and seeds are also reported to contain them in much lower quantities [22-23]. All types of SGs possess a sweetness superiority to sucrose. On average, SGs are 250-300 times sweeter than sucrose, which means even a small
Table 1: Major steviol glycosides of *Stevia rebaudiana*

<table>
<thead>
<tr>
<th>Glycosides</th>
<th>R1</th>
<th>R2</th>
<th>Dry weight (%)</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stevioside</td>
<td>Glcβ1−</td>
<td>Glcβ(1−2)Glcβ1−</td>
<td>5-10</td>
<td>C₃₂H₵₀O₁₃</td>
</tr>
<tr>
<td>Rebaudioside A</td>
<td>Glcβ1−</td>
<td>Glcβ(1−2)[Glcβ(1−3)]Glcβ1−</td>
<td>2.5</td>
<td>C₄₄H₷₀O₂₃</td>
</tr>
<tr>
<td>Rebaudioside B</td>
<td>H</td>
<td>Glcβ(1−2)[Glcβ(1−3)]Glcβ1−</td>
<td>-</td>
<td>C₃₈H₶₀O₁₈</td>
</tr>
<tr>
<td>Rebaudioside C (dulcoside B)</td>
<td>Glcβ1−</td>
<td>Rhaα(1−2)[Glcβ(1−3)]Glcβ1−</td>
<td>1</td>
<td>C₄₄H₷₀O₂₂</td>
</tr>
<tr>
<td>Dulcoside A</td>
<td>Glcβ1−</td>
<td>Rhaα(1−2)Glcβ1−</td>
<td>0.5</td>
<td>C₃₈H₶₀O₁₇</td>
</tr>
<tr>
<td>Rebaudioside D</td>
<td>Glcβ(1−2)Glcβ1−</td>
<td>Glcβ(1−2)[Glcβ(1−3)]Glcβ1−</td>
<td>0.2</td>
<td>C₄₄H₷₀O₂₈</td>
</tr>
<tr>
<td>Rebaudioside E</td>
<td>Glcβ(1−2)Glcβ1−</td>
<td>Glcβ(1−2)Glcβ1−</td>
<td>0.2</td>
<td>C₄₄H₷₀O₂₃</td>
</tr>
<tr>
<td>Rebaudioside F</td>
<td>Glcβ1−</td>
<td>Xylβ(1−2)[Glcβ(1−3)]Glcβ1−</td>
<td>0.2</td>
<td>C₃₈H₶₀O₂₂</td>
</tr>
<tr>
<td>Steviolbioside</td>
<td>H</td>
<td>Glcβ(1−2)Glcβ1−</td>
<td>0.1</td>
<td>C₃₂H₵₀O₁₃</td>
</tr>
</tbody>
</table>

amount of them are sufficient to sweeten any product [24]. Another consumer-attracting property of SGs is having no calories. These molecules are poorly absorbed in the digestive tract. Studies showed that they are completely hydrolyzed to their aglycon steviol but the human intestinal microflora was not able to degrade steviol, which makes SGs zero-calorie sweeteners [25].

**Anti-diabetic Activity**

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin. Insulin is a hormone that regulates blood sugar. Hyperglycaemia, or raised blood sugar, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels [26].

Curi et al. [27] studied the effect of aqueous extracts of *S. rebaudiana* leaves on a glucose tolerance test in 16 healthy volunteers. They administered 5 grams aqueous extract of leaves to volunteers at regular 6-h intervals for 3 days, performed a glucose tolerance test before and after the administration. *S. rebaudiana* extract was reported to increase glucose tolerance and significantly decrease plasma glucose levels in volunteers [27]. More recently, Assaei et al. [28] performed an analysis of...
the hypoglycemic effect of aquatic extract of *S. rebaudiana* in pancreas of diabetic rats. In the study, researchers divided Sprague-Dawley rats into four groups including a normoglycemic group and three diabetic groups in which, one wasn’t treated, one was treated with aquatic extract of *Stevia* (400 mg/kg) and one with pioglitazone, a common used drug with anti-hyperglycemic effect, (10 mg/kg) for the period of 28 days. According to the results, aquatic extract of *Stevia* significantly increases the insulin level and shows anti-hyperglycemic effects [28]. Jeppensen et al. [29] evaluated the potential anti-hyperglycemic effects of stevioside in type 2 diabetic Goto-Kakizaki (GK) rats and in Wistar rats. Their results revealed that in GK rats, stevioside may be responsible for increase of insulin secretion by inducing the genes involved in glycolysis. In Wistar rats, stevioside was found to enhance insulin levels above basal, without altering the blood glucose response. Hence, the researchers concluded that stevioside demonstrates anti-hyperglycemic, insulinotropic and glucagonastatic effects, may be useful in treatment of type 2 diabetes [29]. Geeraert et al. [30] found that stevioside treatment in obese insulin-resistant mice (12-weeks-old) shows an improvement in insulin signaling and antioxidant defense in both the adipose tissue and the vascular wall and an inhibition of atherosclerotic plaque development. Their results also pointed out that stevioside has no effect on weight and triglycerides [30]. Different SGs were also examined for their roles against diabetes in various studies. A study which investigates the potential anti-hyperglycaemic effects of rebaudioside A in isolated mouse islets was conducted by Abudula et al. [31], demonstrated that rebaudioside A potentially stimulates insulin secretion in a dose-, glucose- and Ca²⁺ dependent manner. Another study by the same group evaluated mechanism underlying the insulinotropic action of rebaudioside A in isolated mouse islets. Their findings showed that rebaudioside A contributes in events that are important for glucose-stimulated insulin secretion, such as indirect inhibition of the K⁺-ATP channels [31].

### Anti-hypertensive Activity

High blood pressure, known as hypertension, is a serious medical condition in which the long-term force of the blood against your artery walls is high enough that it may eventually cause cardiovascular problems, such as stroke, heart disease and peripheral arterial disease [32]. Animal studies have shown a blood pressure reducing activity of *Stevia*. Chan et al. [33] investigated the effect of intravenously administrated stevioside on blood pressure of spontaneously hypertensive rats (SHR). They reported a significant hypotensive effect of stevioside (at an intravenous dose of 50, 100 or 200 mg/kg b.w.) on systolic and diastolic blood pressure without any important change in serum dopamine, norepinephrine, and epinephrine levels [33]. Another study conducted by the same group [34] examined hypotensive activity of stevioside in humans (with 106 hypertensive volunteers), revealed a significant decrease in blood pressure after 3 months and persisting throughout the study. Additionally no adverse effects were observed [34]. A study revealed antihypertensive effect of stevioside through inhibiting calcium influx [35]. Results demonstrated that stevioside (25 mg/kg) relaxed the vasopressin-induced vasoconstriction in rats with no effect on phenylephrine and KCl- induced phasic vasoconstriction [35]. In their study with anesthetized dogs, Liu et al. [36] also reported that stevioside possesses antihypertensive activity and its hypotensive mechanism was due to inhibition of the Ca²⁺ influx. According to Hsieh et al. [37], stevioside caused a significant decrease in blood pressure. In their 2-year placebo-controlled study, results showed that 1500 mg (500 mg capsules, 3 times) daily oral stevioside decreased systolic and diastolic blood pressures without any adverse effects, in patients with mild hypertension [37]. Effect of *Stevia* extract intervention on lipid profile was investigated by Sharma et al. [38]. They examined the effects of the extract consumption on 20 hypercholesterolemic women (40-60 years) and found that the consumption of *Stevia* extract (20 mL extract (165 mg/mL) in a 200 ml glass of water) reduced the levels of cholesterol, triglyceride, LDL-C significantly while increased the HDL-C level [38].
**Anti-diarrheal Activity**

Diarrhea describes an increase in the volume and weight of daily stool. The frequency of bowel movements is usually increased as well [39]. Intestinal infections of bacteria and viruses are the most common cause of the disease. These pathogens can cause either direct invasive damage to the intestine or deranged intestinal functions, resulting in diarrheal symptoms [40]. Potential anti-diarrheal effect of stevioside was examined by Tomita et al. [41]. Researchers observed antibacterial effects of hot water extract of *Stevia rebaudiana*. They were the first to report a bactericidal activity of the extract against a broad range of food-borne pathogenic bacteria, including enterohemorrhagic *Escherichia coli*, known to cause severe hemorrhagic/exudative diarrhea. SGs are the main secondary metabolites in *S. rebaudiana* leaves. Thus, they are thought to play a role in anti-diarrheal activities of the extract [41]. Another study [42] reported that a large compound containing polysaccharide in the *Stevia* extract inhibits rotavirus growth. Additionally, some other compounds in the extract were found to possess anti-rotavirus properties [42]. Shiozaki et al. [43] reported inhibitory effects of stevioside on intestinal smooth muscle contraction. Stimulation of this tissue results in hypermotility-associated diarrhea. Researchers have found that stevioside inhibits CaCl$_2$ induced contraction of isolated guinea pig ileum by 40%. Hence, stevioside may be used as a pharmacological compound in the treatment of intestinal hypermotility related diarrhea [43]. Recent studies have reported effects of stevioside and its metabolite, steviol, on ion transportation in different tissues, such as the pancreas, intestine and kidney [39]. This effect of steviol and its analogs on intestinal secretion of Cl$^-$ was studied in human T84 epithelial cell line by Pariwat et al. [44]. Results demonstrated a potential therapeutic effect of steviol and its analogs in the treatment of secretory diarrhea.

**Anti-tumor Activity**

Cancer is a group of diseases caused by loss of cell cycle control. Cancer is associated with abnormal, uncontrolled cell growth. Cancer is generated by both external factors (tobacco, chemicals, radiation, and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism) [45]. Various studies have supported the anti-tumor activity of stevio glycosides, mainly stevioside. In early studies, researchers found the activity of stevioside is similar to many triterpenoids like pachymic acid, 3-O-acetyl-16a-hydroxytrametlenolic acid, poricoic acid B and heliantriol C on tumor promotion by 12-O-tetradecanoyl-phorbol-13-acetate (TPA) [46-47]. Yasukawa et al. also reported inhibitory effect of stevioside tumor promotion by TPA in two-stage carcinogenesis in mouse skin [48]. Bookaewwan et al. [49] examined the effects of stevioside and its metabolite, steviol, on human colon carcinoma cells. Their results showed that steviol plays a role in stimulation of Cl$^-$ secretion and attenuation of TNF-alpha stimulated IL-8 production in colon [49]. A study conducted by Takasaki et al. [50] also evaluated the anticancer effects of stevioside and six related compounds, including the aglycones steviol and isosteviol. Results demonstrated that steviol glycosides and aglycones reduce tumor formation in the two-stage mouse skin carcinogenesis model following sequential exposure to 7,12-dimethylbenz[a]anthracene (DMBA) and TPA [50]. Another study reported these compounds inhibit tumor promotion by blocking Epstein-Barr virus early antigen (EBV-EA) induction [51]. Rajesh et al. [52] evaluated the anti-cancer activity of an ethanolic extract of leaves of *S. rebaudiana* in rats by induced Erlisch’s ascites carcinoma. The ethanolic extract of leaves reduced the tumor volume as well as viable and non-viable tumor cell count [52]. Studies mentioned above show the potential anti-cancer effects of steviol glycosides. Further investigation is needed to enlighten the details of the mechanism of action and to identify anti-tumor aspects.

**Antioxidant Activity**

Antioxidants are compounds that interact with and neutralize free radicals. With this ability, they prevent oxidative damage caused by free radicals [53]. Antioxidants are also known as “free radical scav-
engers.” Human body makes some of the antioxidants, endogenous antioxidants, to neutralize free radicals. Nevertheless, the body relies on external sources, exogenous antioxidants, to obtain the rest of the antioxidants needed. Oxidative stress caused by free oxygen radicals is known to involve in several pathological diseases, such as diabetes, inflammation, aging and cancer. Many of the biologically active compounds in plants are known to possess potential antioxidant activities. Recently, these dietary natural antioxidants have gained much attention in order to prevent the diseases mentioned above [54]. Ghanta et al. [55] studied the oxidative damage-prevention activity and antioxidant potential of S. rebaudiana. Their results showed that at 0.1 mg/mL, the ethyl acetate extract (EAE) of the crude 85% methanolic extract (CAE) of S. rebaudiana leaves inhibited DNA strand scission by •OH, generated via a Fenton reaction, on pBluescript II SK (−) DNA. Its efficacy was better than that of quercetin. The radical-scavenging capacity of CAE was evaluated by the DPPH test (IC₅₀ = 47.66 ± 1.04 μg/mL). EAE derived from CAE scavenged DPPH (IC₅₀ = 9.26 ± 0.04 μg/mL), ABTS+ (IC₅₀ = 3.04 ± 0.22 μg/mL) and •OH (IC₅₀ = 3.08 ± 0.19 μg/mL). Researchers concluded that S. rebaudiana may be useful as a potential source of natural antioxidants [55]. Phansawan and Pounghangpho [56] studied the antioxidant capacities of five different medicinal plants; Pueraria mirifica, S. rebaudiana Bertoni, Curcuma longa Linn., Andrographis paniculata (Burм.f.) Nees. and Cassia alata Linn. The method was based on inhibition in absorption of ABTS (2,20-azinobis(3-ethyl-benzothiazolline-6-sulphonic acid) technique and the antioxidant capacity was recorded as TEAC. The medicinal plants were subjected to extraction with five solvents including ethanol, methanol, acetone, acetic acid, and distilled water, where the highest antioxidant capacity was found in S. rebaudiana Bertoni [56]. Shukla et al. [57] evaluated in vitro antioxidant activity and total phenolic content of ethanolic leaf extract of Stevia rebaudiana Bertoni. In the study, the DPPH activity of the extract (20, 40, 50, 100 and 200 μg/ml) was increased in a dose dependent manner, which was found in the range of 36.93-68.76% as compared to ascorbic acid 64.26-82.58%. The ethanolic extract was also found to scavenge the superoxide generated by EDTA/NBT system. The ethanolic extract also inhibited the hydroxyl radical, nitric oxide, superoxide anions with IC₅₀ values of 93.46, 132.05 and 81.08 μg/ml, respectively. Their results clearly indicated that S. rebaudiana has a significant potential to use as a natural antioxidant agent [57]. Kim et al. [58] investigated the antioxidant activity and the bioactive compounds found in water extracts taken from S. rebaudiana leaves and calli. Their results showed that the leaf extracts contained higher amounts of free radicals, hydroxyl radicals and superoxide anion radical scavenging activities than those of the callus extracts [58]. Safety of S. rebaudiana extract and pure SGs has been extensively studied. Various studies have been conducted to investigate toxicity, carcinogenicity and reproduction and developmental safety of Stevia. Toxicity of stevioside and rebaudioside A were examined by many groups. Chickens, rats and hens were tested for different concentrations of stevioside, 96%, %95.6 and 96%, respectively, in various studies [59-60]. Results indicated that stevioside has no oral toxicity [59-60]. Subcronic toxicity of high concentration rebaudioside A was tested in rats [61-62]. Findings showed that rebaudioside A is a non-toxic compound [61-62]. High concentrations of the sweetener rebaudioside A, administered in the diet of rats over 90 days, were not associated with any signs of toxicity [63]. Many in vivo and in vitro carcinogenicity studies and reports have shown that there is no significant evidence of genetic toxicity of SGs. Mutation, chromosome alteration and simple DNA breakage tests detected no damage in genetic material in long and short term exposure to stevioside and rebaudioside A [64]. A report from Food and Agriculture Organization/World Health Organization’s Joint Expert Committee on Food Additives (JECFA) stated that stevioside and rebaudioside A have not shown any evidence of genotoxicity in vitro or in vivo, concluded that SGs do not pose a carcinogenic risk [65]. The report also concluded that steviol (or some of its metabolites) show signs of genotoxic activity in vitro, but produce no significant genotoxicity in vivo up to doses greater than 2000 mg/kg body weight/day [65]. Exposure of stevioside (4 mg/ml in drinking water) for 45 days causes DNA

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Steviol Glycosides from Stevia rebaudiana Bertoni

Esen F.N. . 2016

strand breaks in Wistar rats [66]. However several genetic toxicology experts identified a number of methodological and data interpretation problems, pointed out that the results are flawed [67-68]. Another study revealed positive results for mutagenicity of stevial in mammalian cell assay [69]. Nevertheless, some scientists concluded that stevial genotoxicity in mammalian cells is limited to in vitro tests that may be affected by excessive concentrations of the compound [64]. Stevia has been report-edly used as an oral contraceptive by women from Paraguayan Matto Grosso Indian tribes [4]. This use has led to an interest in the effect of Stevia extracts on reproduction and fertility. An early study has raised concerns about antifertility of stevioside, after Stevia decoctions was reported to decrease livebirth rate in rats [70], but these results could not be replicated [71]. A number of studies carried in mice [72], rats [73-74] and hamsters [75] demonstrated that oral stevioside has no effect on fertility. High purity rebaudioside A was also tested for re-productive safety. Investigation of two-generation reproduction toxicity in rats showed that rebaudioside A has no adverse effects on reproductive performance parameters, including mating-performance, fertility, gestation lengths, estrous cycles or sperm motility, concentration, or morphology [76]. Potential allergenic properties of highly purified SGs are examined in different studies [77-78]. Reports of Stevia hypersensitivity, even to crude extracts, are very rare [79]. During the regulatory re-views of high-purity SG sweeteners, the potential for hypersensitivity was dismissed or minimized by every national and international food safety authority [79]. Recently, S. rebaudiana Bertoni has gained a great importance in food, cosmetic and pharmaceutical industries for its sweet diterpene glycosides [80-84]. SGs have some industrially im-portant features. Non-calorific properties make them very attractive sugar substitutes in food and beverages [24]. Due to low glycemic index and low calorie features, industrial products with SGs mainly aim diabetic and obese customers. However, individuals who prefer whole-organic or low-calorie foods can benefit from Stevia products [85]. Furthermore, various studies have reported that SGs are non-cariogenic sweeteners [86-88], which is another advantage for use in different sweet products such as beverages, candies, chocolates, biscuits, chewing gums and toothpaste. SGs are heat-stable up to 200 °C, acid-stable, not fermentable and does not undergo any browning reaction [84], which make them convenient for the use in the bakery industry. All kind of cooked and baked products such as cakes, breads, pastries can be sweetened with SGs [89]. Additionally, Stevia sweeteners possess high water absorption capacity and high tendency of fat absorption. Sweetness caused by SGs last 40 seconds longer in comparison to table sugar [90]. Organoleptic characteristics were also found acceptable for use as sucro substitute [91]. Because of the properties mentioned above, Stevia and its glycosides are extensively used in foods and beverages. Nowadays, the number of countries accepting the application of Stevia in food and beverages is increasing. Majority of food standards regulating agencies have confirmed that Stevia is safe for use as a general purpose sweetener. In Japan, where artificial sweeteners are banned, authorities accepted the commercial use of Stevia in 1970s. Following Japan, China approved Stevia as food additive in 1984 [92]. As of today, these two countries are the biggest markets for production and utilization of Stevia products [93]. 2008 was a big year for Stevia industry. In this year, United States Food and Drug Administration (FDA) granted permission for use of pure SGs (95%), stated that they are safe for hu-man consumption. Many countries approved the use of Stevia extract and SGs, such as Australia, New Zealand, Russia, Philippines, Argentina and Turkey. Moreover, JECFA regarded the consumption of S. rebaudiana as safe, established an “Acceptable Daily Intake (ADI)” for SGs of 0–4 mg/kg/bw (both for children and adults) [93]. In 2011, European Food Safety Authority (EFSA) au-thorized the use of pure SGs (95%) in foods and beverages across the European Union [94]. In 2015, it is reported that Europe has seen the most dramatic growth rate of Stevia plant over the five years, with a 149% increase [94]. Many interna-tional companies use Stevia in their products. Most of the marketed items primarily contains stevioside and rebaudioside A [96]. Coca-Cola and Cargill Inc. have jointly developed and commercialized a high purity rebaudioside A sweetener, Rebiana, in

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Steviol Glycosides from *Stevia rebaudiana* Bertoni

Esen F.N.  . 2016

2008 [97]. It is the primary source of sweetness in Truvia sweetener brand [98]. Truvia is a sugar substitute which is classified as a natural sweetener by Cargill. It is made of rebiana, erythritol and natural flavors [98]. However the company has settled lawsuits alleging deceptive marketing of Truvia, claiming that erythritol in Truvia is made by genetically modified corn, thus it cannot be classified as natural. In 2015, the company agreed to fund the $6.1 million settlement for distribution to consumers who purchased the product anytime during a six-year period, from early 2008 until July 2014 [99]. Truvia is currently used in yogurts, chocolate bars, ice-cream and various beverages [100]. Over 45 products distributed by Coca-Cola use SGs. One of the most important items is low-calorie drink, Coca-Cola Life. It was launched in Argentina in 2013 and it is currently sold in 27 countries, including USA, UK, Germany, South Africa and Japan [101]. PureVia brand is another example for *Stevia*-based sugar substitutes. It was developed and commercialized by PepsiCo Inc. and Whole Earth Sweetener Company in 2008. Similar to Truvia, it contains high purity rebaudioside A [102]. PureVia is sold in liquid and powder forms and used in various PepsiCo beverages. The brand is labeled as “natural” and it isn’t a product of bioengineering [103]. Recently, international corporations have focused on biotechnological production of SGs. Cargill and Evolva announced their development project to produce fermentation-based SGs in late 2013. By 2014, companies have filed patent applications of fermentative production of rebaudioside M [104]. FDA has issued a GRAS (generally recognized as safe) letter for the new brand, EverSweet, which is defined as “next-generation” rebaudioside D and M sweetener. EverSweet will be launched commercially in 2016 [105]. *S. rebaudiana* Bertoni is an ancient plant with great potential as an agricultural crop for the production of a high-potency natural sweetener. Its sweet phytochemical constitutes, steviol glycosides (SGs) also possess potential health benefits. Biological activities and safety of SGs are summarized in Table 2. Further research needs to be conducted to investigate interactions of SG metabolites with food components. Owing to their industrial properties, SGs are also suitable components for application in food and beverages. It can be concluded from the review that SGs are safe and can be used to improve human health and nutrition in various industries.

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Table 2: Summary of biological activities and safety aspects of steviol glycosides

<table>
<thead>
<tr>
<th>Component</th>
<th>Biological Activity</th>
<th>Safety Aspects</th>
<th>References</th>
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<tbody>
<tr>
<td>Stevioside</td>
<td>Anti-hyperglycaemic</td>
<td>Non-toxic</td>
<td>29, 60</td>
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<tr>
<td></td>
<td>Anti-hypertensive</td>
<td>Non-mutagenic</td>
<td>33, 65</td>
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<td></td>
<td>Anti-diarrheal</td>
<td>No adverse effect on fertility</td>
<td>41, 75</td>
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<td>Anti-tumor</td>
<td>Non-allergenic</td>
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<td>Non-mutagenic</td>
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<td></td>
<td>No adverse effect on fertility</td>
<td>76</td>
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<td></td>
<td>Non-allergenic</td>
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<td>Stevia extract</td>
<td>Anti-hyperglycaemic</td>
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<td>28, 65</td>
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<td>Anti-tumor</td>
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<td>Antioxidant</td>
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**References**


Steviol Glycosides from *Stevia rebaudiana* Bertoni


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