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Review Article

STEVIOL GLYCOSIDES AND THEIR USE IN FOOD PROCESSING: A REVIEW

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ABSTRACT

Steviol glycosides are the proteinacious secondary metabolites present in the leaves of Stevia rebaudiana Bertoni. These SG's are the principal sweetening agents which are 200-300 times more sweeter than sucrose. A number of SG's are present in the leaves of Stevia namely, stevioside, dulcoside A, rebaudioside A, rebaudioside B, rebaudioside C (previously known as dulcoside B), rebaudioside D, rebaudioside E and steviolbioside, 100–125. At the 63rd meeting a temporary ADI of 0-2 mg/kg bodyweight and day, expressed as steviol, was established based on a No-Observed-Effect-Level (NOEL). Steviol glycoside extracts have broad applications as sweetener in the manufacture of fruit and milk drinks, desserts, yoghurt, delicacies, confectioneries, fruit products, processed seafood products, pickles, table-top sweeteners and dietary supplements. Other aspects considered are physicochemical and biologic properties for food processing, stability and therapeutic value of SG's. nutritional information, Stevia market and household uses.

Keywords: Stevia rebaudiana, Steviol glycosides, stevioside and rebaudioside.

INTRODUCTION

Stevia rebaudiana, a perennial herb from the Asteraceae family, is known to the scientific world for its sweetness and steviol glycosides (SGs). Stevia is a genus of about 240 species of herbs and shrubs in the sunflower family (Compositae) native to subtropical and tropical South America and Central America. The species Stevia rebaudiana Bertoni, commonly known as sweetleaf, sweet leaf, sugarleaf, or simply stevia, is widely grown for its sweet leaves. Stevia species are found in the wild in semi-arid habitats ranging from grassland to mountain terrain. Today, stevia is cultivated in many countries around the world, including Latin America and Asian countries. China is the world's largest exporter of steviol glycosides. One of the advantages of stevia is that it can be grown almost anywhere. Its native conditions are sub-tropical, but it is cultivated in both temperate and in tropical areas.

Steviol glycosides

SGs are the secondary metabolites responsible for the sweetness of Stevia. They are synthesized by SG biosynthesis pathway operating in the leaves. Steviol glycosides are tetracyclic diterpenes derived from the same kaurenoid precursor as gibberallic acid **(Bradle and Telmer, 2007)**. Out of various SGs, stevioside and rebaudioside A are the major metabolites. SGs including stevioside have also been synthesized by enzymes and microbial agents **(Yadav and Guleria, 2012)**.

Steviol glycosides are of interest due to their sweetening properties, and stevioside and rebaudioside are the principle components of them. The product is obtained from the leaves of *Stevia rebaudiana* Bertoni. The leaves are processed with hot water and the aqueous extract is further concentrated and purified. The final product may be spray-dried. Stevia extracts generally contain a high percentage of the glycosides stevioside and rebaudioside A, and smaller amounts of other steviol glycosides i.e rebaudioside C, dulcoside A, rubusoside, steviolbioside, and rebaudioside B. The composition of the extracts depends on the composition of the leaves, influenced by soil and climate, and on the extraction and purification processes used. The impurities occurring in extract of the Stevia leaves are typical plant materials, such as pigments and saccharides. **Ray, 2008** reported the proportion in terms of dry weight basis of the four major steviol glycosides found in the *Stevia* plant tissue are 9.1% stevioside, 3.8% rebaudioside A, 0.6% rebaudioside C and 0.3% dulcoside. The two primary compounds, stevioside and rebaudioside A, use only glucose: stevioside has two linked glucose molecules at the top hydrogen site, where rebaudioside A has three, with the middle glucose of the triplet connected to the central steviol structure. The sweetness of rebaudioside increases with increasing amount of sugar units bonded to the aglycon (steviol). However their content in the plant material decreases at the same time (**Kovylyaeva, 2007**)

Chemical names of SG's

Stevioside: 13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester

Rebaudioside A: 13-[(2-O-β-D-glucopyranosyl-3-O-β-Dglucopyranosyl-β-Dglucopyranosyl)oxy]kaur-16-en-8-oic acid, β-D-glucopyranosyl ester

Rebaudioside B: 13-[(2-O-α-L-rhamnopyranosyl-3-O-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester

Dulcoside A: 13-[2-O-α-L-rhamnopyranosyl-β-D-glucopyramosyl]oxy]kaur-16-en-18-oic acid,β-D-glucopyranosyl ester

The diterpene known as Steviol is the aglycone of *Stevia*'s sweet glycosides, which are constructed by replacing steviol's bottom hydrogen atom with glucose (forming an ester), and replacing the top hydrogen atom with combinations of glucose and rhamnose. It is formed by the enzymatic hydroxylation within the plant (**Kim et al., 1996**). The sterol fraction contains the following sterols: stigmasterol (45,8%), beta-sitosterol (39,4%) and campesterol (13,1%), **(D'Agostino and others 1984)**.

The leaves of Stevia rebaudiana bertoni accumulate at least eight Steviol glycosides, the concentrations of which ary quite widely depending on the genotype and production environment (**Kinghorn and Soejarto, 1985; Bradle et al., 1998**, **Starratt et al., 2002**). The diversity of SG's results from elaboration of the aglycone Steviol by various glycosyltransfereases (**Richman et al., 2005**). As a result of differential glycosyltransferases, each SG has distinctive

organoleptic properties. For example, stevioside is reported to be 143 times sweeter than sucrose on a weight basis, but rebaudioside A is 242 times sweeter (**Kasai et al., 1981**)

The commercially available products under various names (Stevioside, Stevia extracts, Purified Stevia Extract etc.) vary in composition from one manufacture to another with respect to the relative ratios of the steviol glycosides and other constituents. Figure 1 show the structures of stevioside and related compounds. In rebaudioside D and E R1 is composed of 2 b-Glc-b-Glc (2 1). In rebaudioside A, B, C, D, E and F in group R2 an additional sugar moiety is added on carbon 3 of the first b-Glc. In rebaudioside F one b-Glc is substituted for by -b-Xyl

The sweetness of any of the stevia compounds is greater than that of saccharose: rebaudioside A (250–450 times); rebaudioside B (300–350 times); rebaudioside C (50–120 times); rebaudioside -D (250–450 times); rebaudioside E (150–300 times); dulcoside A (50–120 times); and steviolbioside (100–125 times). On average, the sweetness of the steviol glycosides is 250–300 times greater than that of saccharose, with low water solubility and high melting points Stevioside, the most abundant steviol glycoside in the leaf of the plant. Table 1 present the different sweetening components in Stevia leaves

Table1. Sweet component of Stevia Leaf

S.No.	Compound name	R1	R2
1	Steviol	H	H
2	Steviolbioside	H	β-Glc-β-Glc(2 1) →
3	Stevioside	β-Glc	β-Glc-β-Glc(2 1) →
4	rebaudioside A	β-Glc	β-Glc-β-Glc(2 1) → ⇩ β-Glc (3 1)→
5	rebaudioside B	H	β-Glc-β-Glc(2 1) → ⇩ β-Glc(3 1)→
6	rebaudioside C (dulcoside B)	β-Glc	β -Glc-α-Rha (2 1) → ⇩ β –Glc (3 1)→
7	rebaudioside D	β-Glc-β-Glc(2-1)	-Glc-β-Glc (2 1) → ⇩ β –Glc (3 1)→
8	rebaudioside E	β-Glc-β-Glc(2-1)	β -Glc-β-Glc (2 1) →
9	rebaudioside F	β-Glc	β-Glc-β-Xyl (2 1) → ⇩ β-Glc (3 1)→
10	dulcoside A	β-Glc	β-Glc-α-Rha (2 1) →

Starratt 2002 isolated rebaudioside F from Stevia leaves and established its structure by chemical and spectral studies.

Li (2009) isolated a new acylated quercetin glycoside quercetin-3-O-(4'''-O-trans-caffeoyl)-alpha-L-rhamnopyranosyl-(1-->6)-beta-D-galacopyranoside (1), along with luteolin (2), quercetin (3), luteolin-7-O-beta-D-glucoside

(4), apigenin-7-O-beta-D-glucoside (5), quercitrin (6), quercetin-3-O-beta-D-arabinoside (7) and 4,5-di-O-caffeoyl quinic acid (8) from the leaves of Stevia rebaudiana Bertoni.

Physicochemical and biological properties of steviol glycosides

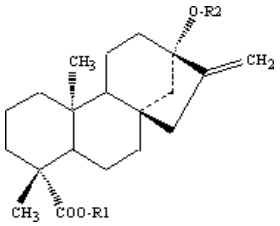

Fig. 1. Structure of Steviol Glycosides

(Source: **Genus *et al.*, 2003**)

The sweetness potency of stevioside has been rated to be 300 times the relative sweetness intensity of 0.4% sucrose solution. The compound exhibits a slightly menthol-like bitter after-taste (**Bakal and Nabors 1986**). The sweetness intensities (i.e. sweetening power relative to sucrose, which is taken as 1) of the other *S. rebaudiana* sweet components have been determined as: dulcoside A, 50–120; rebaudioside A, 250–450; rebaudioside B, 300–350; rebaudioside C (previously known as dulcoside B), 50–120; rebaudioside D, 250–450; rebaudioside E, 150–300; and steviolbioside, 100–125 (**Crammer and Ikan 1987**). Also, stevioside has been found to be synergistic with aspartame, acesulfame-K and cyclamate, but not with saccharin (**Bakal and Nabors 1986**).

The solubility of stevioside in aqueous systems is fairly low but the second most abundant component in *S. rebaudiana* leaves, rebaudioside A, which has a more pleasant taste than stevioside, is 6–7 times more soluble in water, since it contains an additional glucose unit in its molecule (**Kinghorn and Soejarto 1991; Kohda et al. 1976**). Table 2 details the physical properties and chemical index for all the glycosides.

Table 2 Physical properties of steviol glycosides present in *S. rebaudiana*

Compound	CAS number	Molecular weight	Melting point (°C)	Solubility in water (%)
Stevioside	57817-89-7	804	196–198	0.13
Rebaudioside A	58543-16-1	966	242–244	0.80
Rebaudioside B	58543-17-2	804	193–195	0.10
Rebaudioside C	63550-99-2	958	215–217	0.21
Rebaudioside D	63279-13-0	1128	283–286	1.00
Rebaudioside E	63279-14-1	966	205–207	1.70
Steviolbioside	41093-60-1	642	188–192	0.03
Dulcoside A	64432-06-0	788	193–195	0.58

(source: De et al., 2013)

Steviol glycosides are thermostable even at temperatures of up to 200 °C, making them suitable for use in cooked foods (**Lemus-Mondaca, 2012**). **Moraes, 2001** also pointed out that Glycosides are stable to a wide range of pH & heat and are non caloric, non nutritive, non fermentative, prevent caries and dental plaques. Stevioside is a thermally stable molecule upto 100 °C, in the pH range 3–9. However, it decomposes rapidly at higher alkaline pH levels (**Kinghorn and Soejarto 1985**). Both stevioside and rebaudioside A have been found to be stable when formulated in acidulated beverages at room temperatures for a minimum of 3 months (**Chang and Cook 1983**). Solid stevioside is stable for 1 h at 120 °C, and does not undergo browning or caramelisation (**Abou-Arab et al. 2010)**, but decomposes when temperatures exceed 140 °C (**Kroyer 1999**).

Steviol glycosides are thought to possess antioxidant, antimicrobial and antifungal activity.

Safety of steviol glycosides

At the 63rd meeting a temporary ADI of 0-2 mg/kg bodyweight and day, expressed as steviol, was established based on a No-Observed-Effect-Level (NOEL) in a long-term experimental study in rats and applying safety factor of 200, based on the standard safety factor of 100 times an extra factor of 2. The ADI was made temporary and the additional safety factor applied due to lack of data on pharmacological effects of steviol glycosides in humans. JECFA noted that stevioside is being investigated as a potential treatment for hypertension and diabetes, with some evidence of pharmacological effects at higher doses. There was inadequate data to assess whether these pharmacological effects would also occur at lower levels of dietary exposure, which could lead to adverse effects in some individuals. Hence the 63rd JECFA requested additional human studies to address effects in diabetic, as well as normotensive and hypotensive individuals.

The results of specific human studies to address these concerns were evaluated by JECFA at its 68th and 69th meeting (2008). JECFA concluded that the new data were sufficient to allow the additional safety factor of 2 and the temporary designation to be removed. An ADI for steviol glycosides of 0–4 mg/kg bw was established, expressed as steviol. Steviol glycosides are a mixture of compounds with different molecular weights. Since the actual active ingredient is the steviol part of the different molecules, the 0-4 mg refers only to the molecular weight of total steviol in the mixture.

How are steviol glycosides used?

Steviol glycoside extracts have broad applications as sweetener in the manufacture of fruit and milk drinks, desserts, yoghurt, delicacies, confectioneries, fruit products, processed seafood products, pickles, table-top sweeteners and dietary supplements. The extracts are suitable for cooking purposes as they are heat stable, unlike some artificial sweeteners such as aspartame. However, they are unsuitable for certain confectionary such as fudge or icing as they lack bulking property. Water extracts of the crushed leaves of the stevia plant have been used for many years as a sweetener in some countries in South America and Asia. It is also known that stevia leaves are used to prepare a sweetened tea in a number of countries throughout the world.

Dietary Exposures

Steviol glycosides are used to sweeten a number of foods in China, Japan and South America. It is also known that *Stevia* leaves are used to prepare a sweetened tea in a number of countries throughout the world. Broadly speaking stevioside can be used to replace cane sugar or glucoside in most of sugar products. Presently, stevioside is mainly used in drinks and medicine, especially in beverage. It is also used in the frozen food, canned food, candied fruits, spices, wine, chewing gum and toothpaste. The dosage of stevioside varies according to the products. It can be determined through repeated research and experiments so as to guarantee the quality, feeling and taste of product.

The proposed food uses evaluated at the sixty-third meeting of JECFA are shown in Table 4. The additional information on

dietary exposure submitted to the present meeting of the Committee contained proposed additional uses in numerous food categories. The new proposed uses include all those for aspartame use currently approved by the European Union. Table 3 and Table 4 summarizes the proposed uses and use levels, based on the categorization scheme used in the General Standard for Food Additives of the Codex Alimentarius Commission.

Table 3: Food use levels of steviol glycosides reported to the sixty-third meeting of the Committee

Food type	Maximum use level reported (mg/kg)
Beverages	500
Desserts	500
Yogurt	500
Cold confectionery	500
Sauces	1000
Pickles	1000
Delicacies	1000
Sweet corn	200
Bread	160
Biscuits	300

Table 4: Proposed food use levels of steviol glycosides

Food category*	Food use*	Use level (mg/kg)
01.0 Dairy products and analogues, excluding products of food category 02.0	01.1.2 Dairy-based drinks, flavoured and/or fermented (e.g. chocolate milk, cocoa, eggnog, drinking yogurt, whey-based drinks)	*600*
	01.2.1.1 Fermented milks (plain)	1000
	01.7 Dairy-based desserts (e.g. pudding, fruit or flavoured yogurt)	1000
02.0 Fats and oils, and fat emulsions	02.4 Fat-based desserts, excluding dairy-based dessert products of food category 01.7	1000
03.0 Edible ices, including sherbet and sorbet		800
04.0 Fruits and vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweeds, and nuts and seeds	04.1.2.4 Canned or bottled (pasteurized) fruit	1000
	04.1.2.5 Jams, jellies, marmalades	1000
	04.1.2.8 Fruit preparations, including pulp, purees, fruit toppings and coconut milk	1000
05.0 Confectionery	05.1.1 Cocoa mixes (powders) and cocoa mass/cake	1000
	05.1.2 Cocoa mixes (syrups)	1000
	05.1.3 Cocoa-based spreads, including fillings	1000
	05.1.4 Cocoa and chocolate products	2000
	05.2 Confectionery, including hard and soft candy, nougats, etc., other than food categories 05.1, 05.3, and 05.4	1000
	05.3 Chewing gum	5500
	05.4 Decorations (e.g. for fine bakery wares), toppings (nonfruit) and sweet sauces	1000
	Breath-freshening microsweets with no added sugar	6000
	Strongly flavoured freshening throat pastilles with no added sugar	2000
06.0 Cereals and cereal products, derived from cereal grains, from roots and tubers, pulses and legumes, excluding bakery wares of food category 07.0	06.3 Breakfast cereals, including rolled oats	1000
07.0 Bakery wares	07.2 Fine bakery wares (sweet, salty, savoury) and mixes	1700
10.0 Eggs and egg products	10.4 Egg-based desserts (e.g. custard)	1000
11.0 Sweeteners, including honey	11.6 Table-top sweeteners, including those containing high-intensity sweeteners	GMP
12.0 Salts, spices, soups, sauces, salads, protein products (including soya bean protein products) and fermented soya bean products	12.4 Mustards	350
	12.6 Sauces and like products	1000
	12.9.1.1 Soya bean–based beverages	1000

13.0 Foodstuffs intended for particular nutritional uses	13.3 Dietetic foods intended for special medical purposes (excluding products of food category 13.1)	1000 800
	13.4 Dietetic formulae for slimming purposes and weight reduction	(liquid) 600 (solid) 2000
	13.5 Dietetic foods (e.g. supplementary foods for dietary use), excluding products of food categories 13.1–13.4 and 13.6	5500
	13.6 Food supplements	
14.0 Beverages, excluding dairy products	14.1.4 Water-based flavoured drinks, including "sport", "energy" or "electrolyte" drinks and particulated drinks	600
	14.2.7 Aromatized alcoholic beverages (e.g. beer, wine and spirituous cooler-type beverages, lowalcoholic refreshers)	600
15.0 Ready-to-eat savouries	15.2 Processed nuts, including coated nuts and nut mixtures (with, e.g., dried fruit)	500

GMP, Good Manufacturing Practice.

* Food category system (Annex B) of the General Standard for Food Additives of the Codex Alimentarius Commission (Codex Alimentarius Commission, 2007).

(Source: WHO Food Additive Series:60; Safety Evaluation of Certain Food Series)

Stability of SG's in food

Only a limited number of recent studies about the stability of SVGly in food are published in the recent literature (**Clos *et al.*, 2008; Kroyer, 1999; Kroyer, 2010; Prakash *et al.*, 2008; Woelwer-Rieck *et al.*, 2010**). The study by **Clos *et al.* (2008)** deals with the photostability of RebA and Ste in carbonated soft-drinks. Although it is somewhat outside the scope of the present study, it is however relevant, because Clos studied the stability in rather acidic media. Both the acetal group at C13 (as well as the glycosidic bonds between the sugar moieties, which are also acetals) and the ester linkage at C19 may be subjected to hydrolysis at low pH. Clos *et al.* found no indication of decomposition of RebA and Ste after a week of exposure to sunlight at temperatures around room temperature. These results **(Clos *et al.*, 2008)** contradict those of an earlier publication (**Chang & Cook, 1983**). The reason for this discrepancy might be an analysis protocol that was more prone to error than the one that was used by **Clos *et al.* (2008)**

Solid stevioside is stable up to a temperature of 140 °C, for a period of one hour. In aqueous solution, this sweetener is stable in the pH interval between 2 and 10 at a temperature of 80 °C and for a period of up to 4 h (**Kroyer, 1999; Kroyer, 2010**). Kroyer also investigated the effect of several acids on the stability of stevioside. Phosphoric acid has the most pronounced effect: at a concentration of 1 % phosphoric acid, a loss of 30 % is noted after 120 days. **Wölwer-Rieck *et al.* (2010)** compared the stability of Ste and RebA for use in softdrinks. According to their results, RebA is more stable than Ste. Degradation of up to 70 % was observed after 72 h of storage at 80 °C.

In a more general study, the feasibility of high purity RebA for use as a sweetener was investigated (Prakash *et al.*, 2008). In line with the results by Wölwer-Rieck, Prakash *et al.* found RebA to be stable at pH greater than 2. They found no evidence of decomposition when RebA was added to yoghurt or white cake.

Amery et al, 2011, studied the determination of Steviol glycosides in various food categories. The stability of several steviol glycosides was tested in a diverse range of food categories. No sign of decomposition was found under any of the investigated circumstances. So, one can be assured that the addition of steviol glycosides to food will not alter the quality or the normal shelf life of the food.

Therapeautic value of glycosides

Steady consumption of glycoside brings down the level of sugar radionuclides and cholesterol in blood (**Atteh et al. 2008**), facilitates generation of cells and coagulation of blood, reinforces blood vessels and reduces the probability of growth of cancerous cells (**Barriocanal et al. 2008; Jeppesen et al. 2003; Maki et al. 2008; Wingard et al. 1980**). Glycoside exhibits anti-inflammatory (**Jayaraman et al. 2008; Sehar et al. 2008**), diuretic and chloretic properties (**Kochikyan et al. 2006**). It is known to arrest ulcer formation in the gastrointestinal canal (**Kochikyan et al. 2006**) and therefore it is useful in the treatment of diarrhoea (**Chatsudthipong and Muanprasat 2009**). It decreases hypertension and obesity (**Chan et al. 2000; Goyal et al. 2010; Hsieh et al. 2003; Jeppesen et al. 2002; Lee et al. 2001; Pól et al. 2007**), diabetes (**Chen et al. 2006; Jeppesen et al. 2000, 2006)** and activities of human rotavirus (**Suanarunsawat and Chaiyabutr 1997; Takahashi et al. 2001**). It also enhances glucose metabolism (**Suanarunsawat and Chaiyabutr 1997; Toskulkao et al. 1995**) and rejuvenates renal function (**Jutabha et al. 2000**). Glycosides are proven to be useful for treatment of gingivitis (**Blauth de Slavutzky 2010**) and caries effects (**Blauth de Slavutzky 2010; Das et al. 1992; Suanarunsawat and Chaiyabutr 1997).**

CONCLUSION

Steviol glycosides or SG's are the sweetening agents present in stevia leaves. They are heat stable and pH stable. With a number of health benefits steviol glycosides are gaining approval and acceptance among the people as an alternative to sugar.

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