REVIEW

Stevia rebaudiana as a novel source of food additives

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Summary

Stevia rebaudiana is a perennial bush originating in South America, traditionally used as sweetener or as medicine. Stevia contains important natural antioxidants such as flavonoids and various phenolics, tannins, essential oils and other compounds. In addition, stevia could be a potential source of prebiotics, mainly inulin-type fructooligosaccharides with promising beneficial immunomodulating activity. Moreover, stevia is a natural source of steviol glycosides, like stevioside and rebaudioside A. These substances are 200–400 times sweeter than saccharose and are utilized as calorie-free sweeteners, while possibly having also functional properties. Therefore, owing to its important bioactive compounds stevia, may find applications in therapy of various diseases such as diabetes, obesity, aging and dental plaque. In the future, *Stevia rebaudiana* may become increasingly used as a potent source of food additives and nutraceutical ingredients.

Keywords

antioxidants; prebiotics; sweeteners; stevioside; rebaudioside A

Stevia rebaudiana or commonly named stevia is an ancient perennial bush growing up to 1 m tall, which has small elliptic leaves 3–4 cm long [1]. The plant and its sweet taste were first described by the botanist Bertoni [2]. It belongs to *Asteraceae* family and originates in Paraguay and Brazil. Nowadays, due to its unique properties, its cultivation has spread widely throughout America, Asia and Europe [1, 3]. There are more than 230 species in *Stevia* genus but only *S. rebaudiana* is widely grown for its sweet leaves. The taste is caused by sweet steviol glycosides [4], which are formed by enzymatic hydroxylation within the plant [5]. Although the leaves of stevia have the highest contents of various steviol glucosides, these compounds can also be found in declining quantities in the flowers, stems, seeds and roots [6].

For centuries, the leaves of stevia have been used as a sweetener and for the treatment of various ailments [7]. Japan was the first country in Asia by the mid 1970s that used stevia leaves commercially, in the food and drug industries, as an alternative to synthetic sweeteners [8].

NUTRIENT COMPOSITION

As shown in Tab. 1, stevia leaves contain a significant amount of nutrients such as proteins and essential amino acids, lipids, saccharides, vitamins and minerals. The exact composition varies due to regional or climate conditions [3, 9–11].

Stevia leaf oil is considered a good source of monounsaturated fatty acids such as oleic acid (43.6 $g \cdot kg^{-1}$ oil), and polyunsaturated fatty acids such as linoleic acid (93.2–124.0 $g \cdot kg^{-1}$ oil) or α-linolenic acid (215.9–249.5 $g \cdot kg^{-1}$ oil) [12]. Also, the essential oil extracted from stevia

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Tab. 1. Chemical composition of *Stevia rebaudiana* leaves (on dry weight basis).

Nutrient composition	
Crude protein [g·kg^{-1}]	100.0–204.2
Crude fat [g·kg^{-1}]	30.0–43.4
Crude fibre [g·kg^{-1}]	155.2–180.0
Total saccharides [g·kg^{-1}]	352.0–619.3
Ash [g·kg^{-1}]	74.1–131.2
Moisture [g·kg^{-1}]	53.7–70.0
Energy value [kJ·kg^{-1}]	11.3
Water-soluble vitamins	
Vitamin B2 [mg·kg^{-1}]	4.3
Vitamin C [mg·kg^{-1}]	149.8
Folic acid [mg·kg^{-1}]	525.8
Minerals	
Ca [mg·kg^{-1}]	4644–15500
P [mg·kg^{-1}]	114–3500
Na [mg·kg^{-1}]	892–1900
K [mg·kg^{-1}]	17800–25100
Fe [mg·kg^{-1}]	39–555
Mg [mg·kg^{-1}]	3490–5000
Zn [mg·kg^{-1}]	15–64

References [3, 9–11].

leaves contains many important sesquiterpenes (δ-caryophyllene, *trans*-δ-farnesene, humulene) and monoterpenes (linalool, terpinen-4-ol and terpineol) [5, 13]. In addition, some rarely occurring sesquiterpenes like α-longipipene, α-isocomene, modheph-2-ene and (*Z*)-caryophyllene were isolated from the essential oil extracted from the stevia root [14].

Furthermore, stevia leaves and roots contain functional saccharides such as inulin-type fructo-oligosaccharides and dietary fibres, which have been associated with prebiotic, antioxidant and anti-inflammatory effects [1, 15].

On the other hand, stevia leaves contain some potentially antinutritional agents like oxalic acid (23 g·kg^{-1}) and tannins (0.1 mg·kg^{-1}) [3, 10]. Oxalic acid can reduce the digestibility of calcium and other minerals found in leafy vegetables. Tannins have several pharmacological activities i.e. antibacterial and antioxidant but, in high amounts, can limit nutrient digestibility [1, 3, 16].

BIOACTIVE COMPOUNDS AND THEIR POTENTIAL ACTIVITIES

Nowadays, *S. rebaudiana* can be an important functional ingredient with a potential to be used in the food and feed industries, as well as in medicine (Fig. 1).

Antioxidant compounds

Reactive oxygen species and free radicals have been accused for their role in various degenerative and pathological diseases such as cancer, cardiovascular diseases, diabetes and aging. Therefore, nowadays, much attention has been focused on natural antioxidants, due to their ability to neutralize these free radicals with distinct mechanisms of action [17, 18]. These antioxidants can be distinguished into enzymes (superoxidase dismutase, etc.), large molecules (albumin, etc.), small molecules (ascorbic acid, tocopherols, etc.) and others [5, 12, 19, 20]. Many of the antioxidants used in the food industry are synthetic, but recently consumers prefer natural antioxidants.

Stevia leaves can exhibit remarkable antioxidant capacity due to their antioxidant compounds (Tab. 2), mainly phenolics and flavonoids [7, 12, 19, 21–25]. TADHANI et al. [26] reported that stevia leaves contain 25.18 g·kg^{-1} total phenolic com-

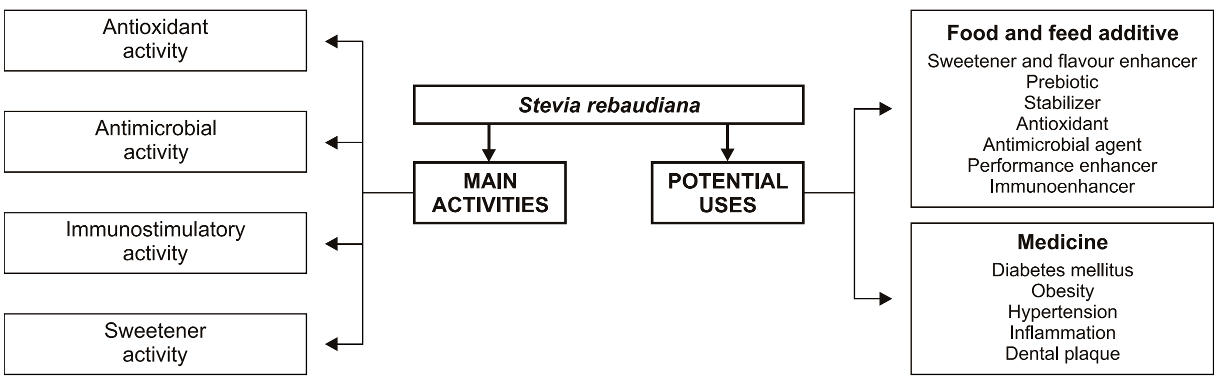

Fig. 1. Activities and potential uses of *Stevia rebaudiana*

pounds and 21.73 g·kg^{-1} flavonoids on dry weight basis. Also, according to Kim et al. [19], the total phenolic compounds of stevia leaves (catechin) were 130.67 g·kg^{-1} of water extract, while the flavonoid content (quercetin) was 15.64 g·kg^{-1} of water extract. Using the ferric reducing ability of plasma assay (FRAP), the total antioxidant capacity of stevia leaves expressed as gallic acid equivalent ranged between 9.66 g·kg^{-1} and 11.03 g·kg^{-1} (on dry weight basis), varying with the used solvent [26]. Stevia leaves also contain other antioxidants like water-soluble vitamin C and minerals such as zinc [12, 19]. In recent years, evidence is mounting that saccharides and saccharide-containing biomolecules such as inulin and stevioside, which are contained in stevia, can be considered as strong antioxidants, because they are capable of scavenging reactive oxygen species [27, 28].

Antimicrobial compounds

Many chemical compounds with potential antimicrobial use are found in stevia leaves: inulin-type fructooligosaccharides, tannins, essential oils and other aromatic substances [3, 15]. Inulins can be isolated from the roots and leaves of stevia, with yields of 4.6% and 0.5%, respectively [15]. Inulins are naturally occurring polysaccharides, categorized as prebiotics, because they can selectively support the growth of beneficial microorganisms in the human and animal gut, while at the same time limiting the growth of potential pathogens [29]. Moreover, inulin consumption can lead to an increase in short chain fatty acids and a decrease in pH in the colon [30], improved mineral uptake [31], reduced blood serum triacylglycerol levels [30, 32], stimulated bifidobacteria proliferation [33], and general enhancement of the performance of the immune system [34].

Tannins are natural polyphenolic compounds of a relatively high molecular weight having the ability to form insoluble complexes with proteins and digestive enzymes, as well as saccharides [35]. They are known to have antidiarrheal and antibacterial properties [36, 37].

Essential oils are plant extracts containing volatile aromatic compounds with antimicrobial and antifungal activities [13, 38]. Essential oils have long been used in medicine and as ingredients of cosmetics [39].

S. rebaudiana and its extracts have antimicrobial properties that can influence the gut microbial populations [40, 41]. Extracts of *S. rebaudiana* have been investigated for its activity against various microorganisms. It was found that these extracts can inhibit in vitro the growth of *Epidermophyton* spp., *Candida albicans* and *Trichophyton mentagrophytes* [42]. Other researchers found that these extracts reduced in vitro the growth of *Salmonella* Typhimurium, *Bacillus subtilis* and *Staphylococcus aureus* [43].

Sweetening compounds

S. rebaudiana leaves contain many diterpene steviol glycosides. All glycosides isolated from this plant have the same steviol (the aglycone of stevioside), but differ in the content of saccharides [44]. Many of these glycosides are natural sweeteners, which are not metabolized by humans and therefore do not provide energy in the diet [3]. The main sweet compounds in *S. rebaudiana* are

Tab. 2. Main antioxidant compounds of *Stevia rebaudiana* leaves.

Phenolic compounds	
Phenolic acids	pyrogallol
	4-methoxybenzoic acid
	4-coumaric acid
	4-methylcatechol
	sinapic acid
	cinnamic acid
Chlorogenic acids	3-caffeoylquinic acid (3-CQA)
	5-caffeoylquinic acid (5-CQA)
	4-caffeoylquinic acid (4-CQA)
	3,5-dicaffeoylquinic acid (3,5-diCQA)
	3,4-dicaffeoylquinic acid (3,4-diCQA)
	4,5-dicaffeoylquinic acid (4,5-diCQA)
	5-caffeoylshikimic acid
	5-feryloylquinic acid
	other chlorogenic acids
Flavonoids	
Flavonols	quercetin
	quercetin-3-O-β-D-arabinoside
	quercetin-3-O-β-D-rhamnoside
	quercetin-3-O-glucoside
	quercetin-3-O-rutinoside
	quercetin-3-O-(4-O-*trans*-caffeoyl)-a-L-rhamno-pyranosyl-(1-6)-β-D-galactopyranoside
	kaempferol-3-O-rhamnoside
Flavones	apigenin
	apigenin-4'-O-β-D-glycoside
	apigenin-7-O-β-D-glycoside
	luteolin
	luteolin-7-O-β-D-glycoside

References [7, 12, 19, 21–25].

stevioside (40–200 g·kg^{-1} of dry weight) and rebaudioside A (30 g·kg^{-1} of dry weight) [6, 27, 45, 46], although their contents vary widely due to the genotype of the plant or due to the cultivation conditions [12, 47]. Besides the major sweetening compounds, stevia contains also other minor diterpene glycosides like rebaudiosides B, C, E, steviobioside, dulcoside A, isosteviol and dihydroisosteviol [48, 49]. Stevia contains also other substances which are not sweeteners, including the triterpenes amyrin acetate and three esters of lupeol, as well as fytosterols stigmasterol, sitosterol and campesterol [5, 50]. According to Wolwer-Rieck [12], new steviol glycosides are still being detected, genuine or no, probably due to the improvements in the purification procedures used. Commercially available glycosides are in crystal, powder, liquid and tablet forms [9].

Stevioside, the major sweet compound of stevia leaves, is a white crystalline powder, highly hygroscopic. It was first isolated in 1931 [51]. The compound can be used as sweetener and flavour enhancer, because it is 200–300 times sweeter than saccharose [52], although it can leave a little bitter aftertaste [53]. This bitterness can be attributed to the presence of sesquiterpene lactones [54] or saccharides, non-glycosidic and unidentified diterpenes/alkaloids present in the leaves [46]. Attempts have been made to improve the palatability of stevioside by adding at least one natural sweetener e.g. saccharose by glycosylation of steviosides, or by supercritical fluid extraction, or through membrane separation [46].

Besides the application as a sweetener, stevioside may have medicinal applications. Stevia leaves and stevioside have been used as substitutes for saccharose in the treatment of diabetes mellitus, obesity, hypertension, inflammation and cancer [27, 28, 55, 56]. Stevioside was examined in vivo with laboratory rats and was found to have immunomodulating effects, to exhibit strong bactericidal effects against pathogenic bacteria, to affect renal function causing hypotension, to reduce blood pressure, to improve insulin sensitivity, to contribute to the gastroprotective effect and to have antiamnesic effects [1, 3, 5, 45, 48, 57].

Rebaudioside A is another natural, zero-calorie, sweet tasting steviol glycoside derived from the leaves of stevia [58]. It is a white to off-white powder, soluble in water. Rebaudioside A has an extra glucose unit compared to stevioside, so it is sweeter (250–400 times more than saccharose) and better in quality, without any undesirable taste characteristics [1, 59, 60]. Prakash et al. [59] reported that rebaudioside A is more stable than aspartame and neotame, which are artificial sweeteners widely used in the food industry. The typical inclusion of rebaudioside A when used as single sweetener in edible products ranged between 50–600 mg·l^{-1} in beverages and 150–6000 mg·kg^{-1} in foods [59].

Since organoleptic characteristics of rebaudioside A are particularly important among the stevia glycosides, some researchers highlighted the need for plant breeders to develop new varieties of *S. rebaudiana*, with a higher content of rebaudioside A, and a reduced content of stevioside [61].

APPLICATION AS A FUNCTIONAL INGREDIENT IN NUTRITION

The stevia glycosides are natural low energy sweeteners, which can be used as functional ingredients in nutrition. Stevia leaves and their extracts can be used by diabetic and phenylketonouric patients as well as by obese people due to the ability to reduce the craving for sweet and fatty foods [62]. Stevioside is very stable at high temperatures up to 200 °C and in a wide range of pH values, is not fermented and does not support formation of plaque on the teeth [63–65]. Accordingly, stevioside has a high degree of stability and can find application as natural stabilizer in a variety of dairy products, beverages, confectionery and other foods [66].

According to Geuns et al. [67], healthy humans who consumed stevioside did not differ from controls in blood pressure and blood biochemical parameters, while no gastrointestinal uptake was detected for stevioside or steviol. Also, no genetic toxicity or mutagenicity was found for steviol glycosides [6, 68]. In acute and subacute toxicology studies in animals it was found that dietary stevioside did not cause any signs of toxicity in rats or hamsters, even at doses up to 2500 mg·kg^{-1} body weight per day [3, 5]. In other studies it was reported that dietary stevioside had not any adverse effect on fertility of mice, rats or hamsters [1].

Additionally, rebaudioside A is stable in a wide variety of foods and beverages such as flavoured ice-tea, juices, flavoured milk and “live” yogurt [59]. Since these products are usually consumed cold, Fry et al. [69] found that rebaudioside A is significantly sweeter under these conditions.

According to Brusick [68], human consumption of rebaudioside A does not pose a risk for genetic damages. Recently in USA, Food and Drug Administration has approved some products containing purified stevia rebaudioside, for example Rebiana, as GRAS (Generally Recognized As Safe) for its use in foods and beverages [70, 71].

Also, the Joint Expert Committee on Food Additives (JECFA) made permanent the acceptable daily intake status for 0–4 $mg \cdot kg^{-1}$ body weight per day (on a steviol basis) [72]. European Food Safety Authority (EFSA) evaluated the safety of steviol glycosides extracted from *S. rebaudiana* leaves as a sweetener and established an acceptable daily intake for humans of 4 $mg \cdot kg^{-1}$ body weight per day. In November 2011, the European Committee (EC) followed the EFSA opinion and authorized steviol glycosides as food additives with the regulation No. 1131/2011 [73].

Regarding the use of *S. rebaudiana* as additive in animal nutrition, the available literature is limited. Some researchers [74] reported that stevia supplemented in newly weaned piglet diets at 83.3 $mg \cdot kg^{-1}$, 167 $mg \cdot kg^{-1}$ and 334 $mg \cdot kg^{-1}$ feed, improved feed/gain ratio and average daily gain of the animals. Other researchers [75, 76] found that dietary supplementation of 0.3% stevia plus 0.3% charcoal in finishing pig diets resulted in higher feed conversion ratio and average daily gain, better immune response, better meat quality and improved meat storage characteristics. On the other hand, the dietary inclusion of 1.76 $g \cdot kg^{-1}$ stevioside in pig diets resulted in decreased feed intake and decreased weight gain [77].

In broiler chickens, the dietary supplementation of 2% dried ground stevia leaves or 130 $mg \cdot kg^{-1}$ pure stevioside during the third and fourth week of age, resulted in higher feed consumption compared to controls, which was converted to higher fat deposition, but without any other positive effects [40]. These results are in agreement with earlier findings in chicken [78], laying hens and meat-type chicken [79].

Dietary stevia leaves or stevioside may have effect on the immune response of broiler chicken, related to some changes in gut microbial populations. It was reported [40, 80] that stevioside fed to mice increased the phagocytic activity, hem agglutination, antibody titre and delayed type hypersensitivity, while other researchers [81] found that stevioside fed to broilers could attenuate the pro-inflammatory response after stimulation of the innate immune response. Moreover, trials with chicken embryos showed that there was no negative effect of the injection of either stevioside (up to 4 mg per egg) or steviol (up to 3 mg per egg) in the egg [62].

CONCLUSIONS

S. rebaudiana contains important natural antioxidants such as flavonoids and various phenolics, tannins, essential oils and other compounds. These substances have antioxidant, antimicrobial, immunostimulatory and sweetening activities. According to recent research, stevia can be used as a novel functional ingredient in the food, feed and medical industry, although further research is needed to confirm all the positive beneficial properties.

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