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STEVIA REBAUDIANA BERTONI – CHEMICAL COMPOSITION AND FUNCTIONAL PROPERTIES

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ABSTRACT

Sweetleaf (*Stevia rebaudiana* Bertoni), currently investigated by many researchers, has been known and used for more than a thousand years indigenous tribes of South America, who called it "kaa-hee" ("sweet herb"). Thanks to its chemical composition and processability sweetleaf may be an alternative for synthetic sweeteners. Nutritional and health-promoting aspects of *Stevia rebaudiana* are presently being studied in many research centres. The aim of this study is to present nutritional and health-promoting value of the still-little known sweetleaf.

Key words: Stevia, Stevia rebaudiana, chemical composition

Stevia is a genus of approx. 200 species of herbs and shrubs from the family Astracae. One of the representatives of the genus Stevia is Stevia rebaudiana, previously named Eupatorium rebaudianum Bertoni (Yadav et al., 2011). Among species from the family Astracae with a sweetening potential (Stevia dianthoidea, S. phlebophylla, S. anisostemma, S. bertholdii, S. crenata, S. enigmatica, S. eupatoria, S. lemmonii, S. micrantha, S. plummerae, S. rebaudiana, S. salicifolia, S. serrata and S. viscida) only Stevia rebaudiana exhibits the highest level of sweetness (Carakostas et al., 2008). Sweetleaf is a perennial plant; however, in areas where temperatures may drop below 0°C it is found as an annual plant. For optimal development it needs a warm climate, abundant rainfall and temperatures of 15-30°C. Sweetleaf plantations require a moist soil of good permeability. Stevia rebaudiana Bertoni is a branched shrub from the family Astracae, originating from South America. It is grown in China, Taiwan, Thailand, Korea, Brazil, Malaysia, Canada, Hawaii and California. It reaches up to 65-80 cm in height, it has oval, lanceolate or spatulate leaves

of 3–4 cm, arranged opposite (Chan et al., 2000). The stem is woody, while the five-petaled flowers are light violet or white.

Stevia rebaudiana Bertoni owes its name to a Swiss naturalist, Moises Santiago Bertoni, who proposed its botanical classification in 1899. Bertoni in his publication of 1918 stressed the health-promoting properties of sweetleaf and its superiority over saccharin used so far. Moreover, he attempted to isolate water-soluble sweetening substances, as well as confirmed its nontoxicity and applicability in the natural form as dried, powdered leaves. The second part of the name is ascribed to a Paraguayan chemist Ovidio Rebaudi, who was the first to isolate from sweetleaf two substances responsible for its sweet taste, including the most important one, named stevioside.

The most important components of *Stevia rebaudiana* include steviol glycosides, which thanks to their high sweetening potential facilitate production of foodstuffs with a reduced energy value. The other components of sweetleaf are of very limited importance due to the very low consumption of this plant.

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	Contents of nutrients in stevia leaves, g 100 g ⁻¹ d.m.					
Component	Tadhani and Subhash (2006)	Goyal and Samsher (2010)	Serio (2010)	Abou-Arab et al. (2010)	Atteh et al. (2011)	
Protein	20.4	11.2	11.2	11.4	16.0	
Fats	4.34	1.9	5.6	3.73	2.6	
Ash	13.1	6.3	-	7.41	15.5	
Carbohydrates	35.2	_	53	61.9	_	
Dietary fibre	_	15.2	15	15.5	6.8	

Table 1. Chemical	composition	of dried stevia	leaves, $g \cdot 100 g^{-1} d.m.$
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– no data.

The chemical composition of stevia leaves changes depending on the degree of their processing. As it results from a study by Snehal and Madhukar (2012), extract from dried stevia leaves contains 10% amino acids, 18% proteins, 33% carbohydrates and 39% reducing sugars, while in the extract from fresh leaves the levels are 25%, 19%, 31% and 25%, respectively. Fat content in dry matter of stevia leaves amounts to 1.9–4.34 g·100 g⁻¹ d.m. (Abou-Arab et al., 2010; Siddique et al., 2014). The chemical composition of this plant is also affected by the method of leaf drying (Gasmalla et al., 2014). Moreover, this plant is a source of vitamins (niacin, thiamine, rutin, ascorbic acid) and minerals (potassium, calcium, magnesium, phosphorus, manganese, silicon, chromium, iron and zinc). The chemical composition of stevia leaves is presented in Tables 1 and 2.

Table 2. Chemical composition of dried stevia leaves depend-ing on the applied drying method (Gasmalla et al., 2014)

Component	Sun-dried	Over-dried	Microwave dried
		%	
Protein	10.73	7.46	4.45
Fats	6.13	4.39	4.18
Ash	12.06	8.06	4.65
Carbohydrates	63.10	69.85	73.99
Dietary fibre	5.03	5.26	4.35
Reducing sugars	4.5	4.8	5.3

PROTEINS

Proteins, peptides and amino acids, being different matrices, are essential cell components. Mohammad et al. (2007) in stevia leaves identified 9 essential amino acids, i.e. glutamic acid, aspartic acid, lysine, serine, alanine, proline, tyrosine, isoleucine and methionine. Abou-Arab et al. (2010) isolated 17 amino acids (Table 3).

CARBOHYDRATES

Carbohydrates are the main sources of energy and they are found as structural components of cellular elements (Lemus-Modaca et al., 2012). Carbohydrate contents in dry leaves of sweetleaf ranged from 35.2 to $61.9 \text{ g} \cdot 100 \text{ g}^{-1}$ product (Abou-Arab et al., 2010; Boonkaewwan et al., 2006). Their positive action is connected with prebiotic properties promoting proliferation of beneficial intestinal microflora. Sweetleaf roots and leaves contain fructooligosaccharides at 4.6% and polysaccharides, which regulate lipid metabolism and control blood sugar level (Braz De Oliveira et al., 2011).

LIPIDS

Lipids are biologically active substances essential for the human organism, storing energy, forming elements of cell membrane structures, regulating physiological functions. Not all fatty acids may be synthesised in the organism, thus their intake with food is required. Dried stevia leaves contain from 1.9 g \cdot 100 g⁻¹ to

Contents of essential amino acids, g·100 g ⁻¹ d.m.					
Amino acid	Abou-Arab et al. (2010)	Li et al. (2011)	Amino acid	WHO data of 2002 (2007)	WHO data of 1985 (2007)
Arginine*	0.45	0.81	Histidine	10	8-12
Lysine	0.70	0.15	Isoleucine	20	10
Histidine	1.13	0.34	Leucine	39	14
Phenylalanine	0.77	0.88	Lysine	30	12
Leucine	0.98	1.30	Methionine + cysteine	15	13
Methionine	1.45	ND	Phenylalanine + tyrosine	25	14
Valine	0.64	0.94	Threonine	15	7
Threonine	1.13	0.75	Tryptophan	4	3.5
Isoleucine	0.42	0.72	Valine	26	10
Total	7.67		Total	184	93.5

Table 3. Amino acid composition of stevia leaves, g 100 g⁻¹ d.m.

*Not considered to be an essential amino acid in the FAO/WHO/UNU report (WHO, 2007).

5.6 g \cdot 100 g $^{-1}$ lipids (Goyal and Samsher, 2010; Serio, 2010). Analysis of fatty acids showed that sweetleaf contains fatty acids: palmitic, linolenic, linolic, oleopalmitic, stearic and oleic acids (Table 4). An adequate intake of unsaturated fatty acids reduces the risk of ischaemic heart disease and enhances immunity.

Table 4. The composition of fatty acids in oil from stevia leaves

	Fatty acid contents, g 100 g-1			
Fatty acids	Tadhani and Subhash (2006)	Atteh et al. (2011)		
Palmitic acid	27.51	29.5		
Oleopalmitic acid	1.27	3.0		
Stearic acid	1.18	4.0		
Oleic acid	4.36	9.9		
Linolic acid	12.40	16.8		
Linolenic acid	21.59	32.6		

VITAMINS AND MINERALS

Vitamins and minerals are essential for the proper functioning of the organism. Their adequate supply promotes e.g. their optimal regulation of hormone levels, growth regulators and differentiation of cells and tissues, protects against oxidative damage. Sweetleaf is a source of folic acid (52.18 mg \cdot 100 g⁻¹), ascorbic acid (14.98 mg \cdot 100 g⁻¹) and slight amounts of vitamins B (Bugaj et al., 2013). Kim et al. (2011) isolated water-soluble vitamins from leaves and calluses of sweetleaf (Table 5).

Stevia leaves also contain such minerals as K, Mg, S, Na, P, Cu, Co, Fe, Mn, Zn, Se and Mo (Boonkaewwan et al., 2006). Kobus-Moryson et al. (2014) showed that extracts from sweetleaf contain considerable amounts of Zn and Cu. Mineral contents in dried leaves are presented in Table 6. Bioavailability of iron and calcium from stevia is limited by the presence of oxalic acid (2295 mg·100 g⁻¹).

Vitamins -	Contents, mg·100 g ⁻¹ d.m.		
vitamins -	leaf	callus	
Vitamin C	14.98	1.64	
itamin B2	0.43	0.23	
itamin B6	0	0	
olic acid	52.18	0.09	
iacin	0	0	
hiamine	0	0	

Table 5. Contents of water soluble vitamins in *Stevia rebaudiana*, mg·100 g⁻¹ d.m. (Kim et al., 2011)

seeds. The primary glycosides in Stevia rebaudiana include steviosides, rebaudiosides A through F, as well as dulcosides. The latest studies conducted by Prakash et al. (2014) showed the presence of rebaudioside M in sweetleaf. This glycoside is 200–350 times sweeter than sucrose, but has a slightly bitter and licorice aftertaste. Each of the glycosides isolated from Stevia rebaudiana has the same steviol stem. The differences are connected with the contents of carbohydrate residues, mono-, di- and tri-saccharides containing glucose and/or ramnose at positions C13 or C19 (Kobus--Moryson et al., 2014). Contents of primary diterpene glycosides in leaf dry matter of *Stevia rebaudiana* are presented in Table 7. Some literature data suggest that

Table 6. Contents of minerals in dried leaves of Stevia rebaudiana, mg·100 g-1

	Contents of minerals, mg·100 g-1					
Minerals	Mishra et al. (2010)	Goyal and Samsher (2010)	Abou-Arab et al. (2010)	Tadhani and Subhash (2006)	Atteh et al. (2011)	
Calcium	464.4	544	17.7	1 550	8.2	
Phosphorus	11.4	318	_	350	2.6	
Sodium	190	89.2	14.93	160	0.7	
Potassium	1 800	1 780	21.15	2 510	17.3	
Iron	55.3	3.9	5.89	36.3	366	
Magnesium	349	349	3.26	_	2.4	
Zinc	1.5	1.5	1.26	6.39	20	

– no data.

DITERPENE GLYCOSIDES

Glycosides are a group of organic compounds, which molecules are composed of the sugar group bonded with an aglycon group. Diterpene glycosides, entkaurene derivatives found in sweetleaf, are responsible for its high sweetening potential. Nine such substances have been isolated to date. Depending on growth conditions, cultivation and tillage techniques their contents range from 4 to 20% fresh leaf weight (Gasmalla et al., 2014; Pól et al., 2007). The levels of glycosides vary in individual plant organs. Leaves are their richest sources, followed by flowers, stems and rebaudioside is not a native component of stevia, but rather a product formed during preparation of the plant to chemical analyses.

STEVIOSIDE

Stevioside accounts for 4 up to 13% all glycosides in stevia. It has a permanent bittery or stringent aftertaste. Comparative organoleptic analyses showed that pure stevioside is 300 times sweeter than sucrose at a concentration of 0.4%, 150 times sweeter than sucrose when matching a 4% sucrose solution and 100 times when matching a 10% sucrose solution (Hojden,

Type of	Contents of c	Sweetening potential		
glycoside	Gardana et al. (2010)	Goyal et al. (2010)	Atteh et al. (2011)	Nabors O'Brien (2012)
Stevioside	5.8	9.1	6.5	150-250
Steviol	-	_	_	_
Steviolbioside	_	_	_	90
Rebaudioside A	1.8	3.8	2.3	200-300
Rebaudioside B	-	_	_	150
Rebaudioside C	1.3	0.6	_	30
Rebaudioside D	_	_	-	221
Dulcoside A	_	0.3	_	30

– no data.

2000). Molecules of stevioside are highly stable in aqueous solutions within a broad range of pH (1–10) and temperatures up to 198°C. Kroyer (2010) showed in his study that steviosides are stable at various processing and storage conditions and in interactions with water-soluble vitamins, organic acids, sweeteners and coffee. During thermal processing they do not participate in the Maillard reactions. Moreover, steviosides do not ferment.

REBAUDIOSIDE

Rebaudioside A is the most important rebaudioside, it is 250–450 times sweeter than sucrose and it is found in *Stevia rebaudiana* at 2–4% leaf dry matter. It is the most stable of glycosides and has no bitter aftertaste, in contrast to steviosides. Rebaudioside A is metabolised by intestinal microorganisms to stevioside and next it is transformed to glucose and a molecule of steviol. Apart from diterpene glycosides, sweetleaf contains also labdane diterpenes and triterpenes.

Analyses of this plant detected such sterols as stigmasterol, beta-sitosterol, campesterol and daucosterol as well as flavonoid glycosides, including apigenin, quercetin, luteolin, kaempferol glycosides (Kochikyan et al., 2006).

ANTIOXIDANTS

Oxidation is an important biological process essential for the production of energy in the human organism. During metabolism molecular oxygen is reduced to water. In the course of electron transfer free reactive oxygen species, such as hydrogen peroxide, hydroxyl and peroxide radicals. Free radicals are considered to be the causative agents in the development of neurological diseases, inflammations, reduced immunity, ageing, ischaemic heart disease, stroke, Alzheimer's and Parkinson's disease as well as cancer (Hou et al., 2003; Parejo et al., 2002). Leaves of *Stevia rebaudiana* Bertoni were found to contain polyphenolic compounds exhibiting antioxidant properties (Table 8; Muanda et al., 2011; Shukla et al., 2009; Tadhani et al., 2007).

Numerical values in Table 8 referring to contents of polyphenols and flavonoids were expressed as gallic acid equivalents, while antioxidant potential was assessed using the FRAP method.

CONCLUDING REMARKS

Stevia rebaudiana Bertoni has an increasingly extensive range of applications worldwide, not only as

	Content, mg/g				
Antioxidants	Soluble in methanol extract (Tadhani et al., 2007)	Soluble in methanol extract (Liu et al., 2003)	Soluble in ethanol extract (Serio, 2010)		
Polyphenols	25.18	25.25	61.50		
	21.73	23.46			
Flavonoids	21.73	23.46	_		

- no data.

a sweetener, but also as a food additive reducing energy value of foodstuffs. Apart from numerous studies confirming health-promoting properties of *Stevia rebaudiana*, as well as its applicability as an adjuvant in the treatment of chronic diseases, further research needs to be conducted to investigate interactions of stevia metabolites with food components and to determine maximum daily intake of this food additive.

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STEVIA REBAUDIANA BERTONI – SKŁAD CHEMICZNY I WŁAŚCIWOŚCI FUNKCJONALNE

STRESZCZENIE

Stevia rebaudiana Bertoni jest obiektem badań wielu współczesnych naukowców. Roślina jest znana od setek lat wśród plemion indiańskich. Mieszkańcy Ameryki Południowej nazywali ją "kaa-hee", co w ich języku oznacza liść miodowy. Skład chemiczny oraz właściwości technologiczne stewii sprawiają, iż może ona być alternatywą syntetycznych środków słodzących. Aspekty żywieniowe i zdrowotne stewii są obecnie przedmiotem badań w wielu ośrodkach naukowych. Praca ma na celu przybliżenie walorów żywieniowych i zdrowotnych stewii.

Słowa kluczowe: stewia, *Stevia rebaudiana*, skład chemiczny

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