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# DIRECTIONS ON THE USE OF STEVIA LEAVES (STEVIA REBAUIDANA) AS AN ADDITIVE IN FOOD PRODUCTS

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#### **ABSTRACT**

Due to the high intake of sugars, especially sucrose, global trends in food processing have encouraged producers to use sweeteners, particularly synthetic ones, to a wide extent. For several years, increasing attention has been paid in the literature to the stevia (*Stevia rebauidana*), containing glycosidic diterpenes, for which sweetening properties have been identified. Chemical composition, nutritional value and application of stevia leaves are briefly summarized and presented.

**Key words:** stevia, *Stevia rebaudiana*, health effect, stevioside, rebaudioside, sweetener

### INTRODUCTION

In recent years, there has been a significant increase in the occurrence of diet-dependent diseases, such as obesity, diabetes, cancer, cardiovascular diseases, and also dental problems, which are a consequence of, among others, inappropriate diet and a lack of physical activity. Due to the high intake of sugars, especially sucrose, global trends in food processing have encouraged producers to use sweeteners, particularly synthetic ones, to a wide extent (Anton et al., 2010). For several years, increasing attention has been paid in the literature to the stevia (*Stevia rebauidana*), 'the sweet herb from Paraguay', a natural alternative to 'white sugar' (Soejarto, 2002; Chatsudthipong and Muanprasat, 2009; Samsheri Goyal and Goyal, 2010; Mishra et al., 2010; Thomas and Glade, 2010; Yadav et al., 2011).

Stevia rebaudiana (Bertoni) belongs to the Asteraceae family and originates from South America; to be precise, from the mountainous region of the Cordillera del Amambay in north-eastern Paraguay near the border with Brazil (Soejarto, 2002; Yadav et al., 2011; Lemus-Mondaca et al., 2012). For centuries, this plant has been locally known by native Guarani Indians as 'kaa he-he', which means 'sweet herb', and has been used in the local cuisine and in the treatment of various ailments (Soejarto et al., 1983; Jeppesen et al., 2002; 2003). The natural environment of *Stevia rebaudiana* are subtropical meadows at an altitude of about 200–500 meters above sea level, with temperatures ranging from –6°C to 43°C, average 23°C (Brandle and Rosa, 1992).

The annual rainfall ranges between 1500–1800 mm (Yadav et al., 2011). The plant reaches a height of 65 cm to even 120 cm, possesses ellipse-shaped leaves and blooms in white. Cultivation conditions for stevia are quite varied. Stevia grows both on dark, damp, sandy soils and on loamy, highly-permeable soils. In addition, it is to be found on the banks of swamps, on infertile, acidic sands or muck soils. The optimal

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subsoil for the cultivation of *S. rebaudiana* is constantly moist, but not exposed to prolonged flooding (Mishra et al., 2010; Lemus-Mondaca et al., 2012). The first attempts to crop outside the natural environment were reported in the UK in 1943, then in 1968 in Japan (Lewis, 1992), and currently the main producers of stevia on a large scale are countries such as Brazil, Korea, Mexico, the U.S.A., Indonesia, Tanzania, Canada and India (Brandle and Rosa, 1992; Fors, 1995; Yadav et al., 2011).

S. rebaudiana Bertoni was botanically classified in 1899 by Moisés Santiago Bertoni. Originally called rebaudianum Eupatorium, in 1905 its name was changed to S. rebaudiana Bertoni (Bertoni, 1905). Undoubtedly, the major advantage of the plant is the presence of steviol glycosides (an overall amount of 4–20% of the dry weight) (Starrat et al., 2002; Ghanta et al., 2007), which provide a sensation of sweetness up to 450 times more intense than that of sucrose (Chatsudthipong and Muanprasat, 2009; Thomas and Glade, 2010). The amount of glycosides in different parts of the plant varies and is presented as follows (in descending order): leaves, flowers, stalk, seeds and roots. Dwivedi claimed that the roots are the only organs that do not contain stevioside; however, the sweetness of the leaves is two twice as high as that in the inflorescence (Dwivedi, 1999; Yadav et al., 2011).

Eight glycosidic diterpenes, for which there are sweetening properties in the tissues of stevia leaves, have been identified (Tateo et al., 1999; Singh and Rao, 2005; Abou-Arab et al., 2010). The most desired glycosides are stevioside (5–10% d.w.) and rebaudioside A (2–4% d.w.) (Dacome et al., 2005; Yadav et al., 2011). Stevia rebaudiana steviosides and related compounds structures presents Figure 1. Stevioside, the main sweetener, which constitutes 60–70% of the total glycoside content present in the leaves and also in lesser amounts in the tissues of Stevia stalks, is 110-270 times sweeter than sucrose (Yadav et al., 2011). In the 1970s, stevioside, as well as stevia extracts, were formally accepted in Japan as a sweetener. At the same time, and as a result of detailed analyses of the glycosides occurring in stevia tissues, rebaudioside A, inter alia, was isolated and described (Barriocanal et al., 2008) as having a sweetening effect even higher than stevioside (250–450 times sweeter than sucrose) (Chatsudthipong and Muanprasat, 2009; Thomas and

3		
Compound	R1	R2
Steviol	Н	Н
Steviolbioside	Н	β-Glc-β-Glc(2 $\rightarrow$ 1)
Stevioside	β-Glc	β-Glc-β-Glc(2 $\rightarrow$ 1)
Rebaudioside A	β-Gle	$\beta$ -Glc- $\beta$ -Glc(2 $\rightarrow$ 1) I $\beta$ -Glc(3 $\rightarrow$ 1)
Rebaudioside B	Н	$β$ -Glc- $β$ -Glc(2 $\rightarrow$ 1) I $β$ -Glc(3 $\rightarrow$ 1)
Rebaudioside C (Dulcoside B)	β-Gle	$\beta$ -Glc-α- Rha(2 $\rightarrow$ 1) I $\beta$ -Glc(3 $\rightarrow$ 1)
Rebaudioside D	$\beta$ -Glc- $\beta$ -Glc(2 $\rightarrow$ 1)	$β$ -Glc- $β$ -Glc(2 $\rightarrow$ 1) I $β$ -Glc(3 $\rightarrow$ 1)
Rebaudioside E	$β$ -Glc- $β$ -Glc(2 $\rightarrow$ 1)	β-Glc-β-Glc(2→1)
Rebaudioside F	β-Gle	$β$ -Glc- $β$ -Xyl(2 $\rightarrow$ 1) I $β$ -Glc(3 $\rightarrow$ 1)
Dulcoside A	β-Glc	β-Glc-α- Rha(2→1)

Glc – glucose, Xyl – xylose, Rha – rhamnose, sugar moieties (De et al., 2013; Geuns, 2003).

**Fig. 1.** *Stevia rebaudiana* steviosides and related compounds structures

Glade, 2010). The compilation of stevia sweetness in comparison with other compounds presents Table 1.

Rebaudioside A levels range between 30–40% of the total content of sweeteners in stevia. Rebaudioside A,

**Table 1.** Stevia rebaudiana leafs and other compounds sweetness level in reference to sucrose

Sweetener		Sweeteness facto (x sucrose)
Aspartame		350–400
Acesulfame K		150-200
Xylitol		0.8-1.1
Lactitol		0.3-0.5
Taumatin ( <i>Thaumato-coccus danieli</i> )		2000–3000
Stevia rebaudiana leafs		15–45
Steviol glycosides		
	Stevioside	200-450
	Steviolbioside	100-125
	Rebaudioside A	150-450
	Rebaudioside B	30–350
	Rebaudioside C	50-450
	Rebaudioside D	150-450
	Rebaudioside E	150-300
	Rebaudioside F	30–120
	Dulcoside A	50-125
	Rubusoside	114

Compilation of references: Kinghorn and Compadre (2001), Chatsudthipong and Muanprasat (2009), Geuns (2003), Das et al. (2007), Gwak et al. (2012), Świąder et al. (2011), Bugaj et al. (2013), Goudable (2011).

in comparison to stevioside, is characterized by a better flavor profile due to the lack of flavor in the mouth, often described as 'liquorice, pungent'. Therefore, the authors of many scientific reports on steviol glycosides pay special attention to the ratio of rebaudioside A to stevioside; namely, the higher its value, the higher the quality of sweetening, while the equalized ratio of the above-mentioned glycosides eliminates any undesirable flavor profile, and the effect of sweetening is similar to sucrose (Yadav et al., 2011).

On March 10, 2010 ANS (The Panel on food additives and nutrient sources added to food) issued a decision on the safety of steviol glycosides, prepared

on the basis of a proposal from the European Commission (EFSA, 2010). The document cites the results of conducted studies, in which it has been found that substances derived from stevia are not carcinogenic or associated with any other negative effects. Furthermore, an acceptable daily intake (ADI - Acceptable Daily Intake) was established at 4 mg per kilogram of body weight. Then, on November 11, 2011, the European Commission passed a regulation allowing the use of steviol glycosides in 31 different food categories, including beverages, desserts, sweets and sweeteners. Previously in Poland, products made of stevia had been sold as a cosmetic for external use. Currently, stevia or products made of stevia are approved for use as a food additive, sweetener (Singh and Rao, 2005; Yadav et al., 2011; Commission Regulation, 2012; Lemus-Mondaca et al., 2012; Elkins, 1997).

## CHEMICAL COMPOSITION AND NUTRITIONAL VALUE OF STEVIA LEAVES

Stevia (*Stevia rebaudiana* Bertoni) is a low-calorie plant, because it provides the body with only 2.7 kcal/g. This means that, due to its sweetening capabilities (steviol glycosides are up to 450 times sweeter than sucrose) and also the calculated energy supply, stevia can compete with currently known and commonly used sweeteners such as acesulfame K (zero calories), aspartame (4 kcal/g), saccharin (zero calories) and sucralose (zero calories) (Savita et al., 2004). This situation results from the fact that stevia decomposes only marginally in the gastrointestinal tract.

Dried stevia leaves are characterized by a sufficiently significant protein content which ranges from 10 to 20.4 g/100 g of the product. Mohammad et al. (2007) have identified nine amino acids in the leaves of stevia; namely, glutamic acid, aspartic acid, lysine, serine, L-isoleucine, alanine, proline, tyrosine and methionine. Then, Abou-Arab et al. (2010) determined a total of seventeen amino acids, and of exogenous amino acids the occurrence of all except for tryptophan was reported, which makes the protein of stevia leaves very valuable from a nutritional point of view. Stevia is characterized by low lipid content in the dry mass, ranging from 1.9 to 5.6 g/100 g of the product. On the other hand, the determined ash content deserves special attention (6.3 to 13.1 g/100 g of the

product). Stevia contains significant quantities of important minerals that are essential to protect an organism, and for the regulation and maintenance of various metabolic processes. Elements such as potassium, calcium, magnesium, sodium, zinc and iron have been determined in stevia leaves. In addition, the results obtained by Kim et al. (2011) on stevia show that 'sweet herb' also constitutes a good source of some vitamins. First of all, stevia is characterized by a high content of folic acid (52.18 mg/100 g of aqueous extract of stevia) and vitamin C (14.98 mg/100 g of aqueous extract of stevia).

## ANTIOXIDANT PROPERTIES AND THE TOTAL POLYPHENOL CONTENT

In recent years, in the literature authors pay increasing attention to antioxidant compounds, due to their ability to neutralize free radicals (Devasagayam et al., 2004; Gramza-Michałowska and Korczak, 2013; Kuźma et al., 2014; Sarić et al., 2013; Bajčan et al., 2013). It has been found that antioxidants may interfere with oxidation reactions with free radicals, exhibit properties to chelate catalytic metals, as well as scavenge oxygen (Buyukokuroglu et al., 2001). Antioxidant compounds present in edible plants are currently promoted as food additives, as they exhibit few or no toxic side effects (Seong et al., 2004).

There are a lot of reports devoted to the phenolic compounds occurring both in the edible and non-edible parts of plants, which are at the same time characterized by a high antioxidant activity. These compounds are important components of plants, necessary for their proper growth and development, and protect them from pests and damage (Jeszka et al., 2010; Kobus et al., 2009; Kucner et al., 2014). Polyphenols play an important role in the prevention of many diet-dependent diseases, among others heart diseases, atherosclerosis or cancer (Sidor and Gramza-Michałowska, 2014). Widely known natural antioxidants are  $\alpha$ -tocopherol and L-ascorbic acid, which exhibit a beneficial effect on the human organism and are reported to be safe. More powerful antioxidants are the synthetic compounds butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) however, they also exhibit possible negative effects on the human organism by activating promoters of carcinogenesis (Seong et al., 2004; Anagnostopoulou et al., 2006; Tadhani et al., 2007; Shukla et al., 2009). Hence, one can observe a growing demand for new, natural antioxidants.

Extracts from the leaves of stevia contain flavonoids, alkaloids, water-soluble chlorophylls and xanthophylls, hydroxycinnamic acids (such as caffeic, chlorogenic), oligosaccharides, free sugars, amino acids, lipids, oils and minerals (Komissarenko et al., 1994). Tadhani et al. (2007) evaluated the *in vitro* antioxidant activity of HCl-methanol leaves and stalks of stevia extracts (Stevia rebaudiana). The total polyphenol content in stevia extracts was estimated at 25.18 and 35.86 mg/g of stevia leaves and callus per gallic acid, respectively. The flavonoid content was 21.73 mg/g in the stevia leaves and 31.99 in the callus (per gallic acid). Moreover, the ferric reducing ability of plasma (FRAP) and radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) tests were also performed to estimate the antioxidant activity. From the conducted studies, one can conclude that extracts from stevia leaves exhibit high antioxidant activity and constitute a good source of antioxidants. Research of Shukla et al. (2009) clearly indicated that stevia plant possess significant potential to be used as promising natural antioxidant.

## STEVIA LEAVES IN THE PREVENTION OF CIVILIZATION DISEASES

Reduction of the risk and/or prevention against the development of diet-dependent diseases can be achieved through a change in lifestyle and eating habits, and the introduction of a well-balanced diet into the nutrition of a population. The reported properties of stevia (*Stevia rebaudiana*) have resulted in significantly increased interest in the therapeutic potential of stevia in food production targeted at the human organism.

The leaves of *stevia rebaudiana* contain low-calorie sweeteners (steviol glycosides), the consumption of which may have a beneficial effect on human health (Gardana et al., 2010). Stevia glycosides possess valuable biological properties. It has been proved that regular consumption of these compounds reduces glucose and cholesterol concentrations (Atteh et al., 2008), enhances the regeneration of cells and blood clotting, inhibits tumor cell growth and strengthens blood vessels (Jeppesen et al., 2003; Barriocanal et al., 2008; Maki et al., 2008). Moreover, it has been noted that the substances

of Paraguay sweet leaves exhibit cholagogic (Kochikyan et al., 2006), anti-inflammatory (Jayaraman et al., 2008; Sehar et al., 2008) diuretic activity, prevent gastrointestinal ulcers (Kochikyan et al., 2006), exhibit antihypertensive (Chan et al., 2000; Lee et al., 2001; Jeppesen et al., 2002), antihiperglycemic (Suanarunsawat and Chaiyabutr, 1997; Jeppesen et al., 2000, 2002; Chen et al., 2006), antirotaviral activity (Suanarunsawat and Chaiyabutr, 1997; Takahashi et al., 2001), exhibit a beneficial effect on glucose (Shivanna et al., 2013; Suanarunsawat and Chaiyabutr, 1997; Toskulkao et al., 1995) and kidney (Jutabha et al., 2000) metabolisms.

### STEVIOL GLYCOSIDES APPLICATION IN FOOD

An increasing intake of sucrose has caused several nutritional and medical problems, including obesity (Anton et al., 2010; Puri et al., 2012). Indeed, there is an alarming increase in the frequency of the incidence of obesity, diabetes type 2 and metabolic syndrome in children and adults worldwide. This is somewhat related to the increasing availability of high-calorie food, including beverages. Therefore, for more than two decades, there has been a very strong increase in interest in sweeteners, which do not introduce additional energy into the organism (Brahmachari et al., 2011; Boudová Pečivová et al., 2013; Reguła and Kowalewska, 2010). On the other hand, consumers increasingly pay attention to the character of the consumed food, whether it fits with the motto of 'natural, healthy food'. Stevia (Stevia reaudiana Bertoni) as the 'miraculous Paraguay herb', providing a sensation of sweetness up to 300 times more intense than sucrose (steviol glycosides), is an ideal natural sugar substitute (Anton et al., 2010; Puri et al., 2012).

In the natural environment, tribes of Indians (Guarani) used 'honey leaves' both in their cuisine, among others for sweetening their yerba mate (Soejarto et al., 1983), as well as in medicine, in the treatment of many skin and mouth diseases etc. (Brandle and Telmer, 2007; Lemus-Mondaca et al., 2012).

In Japan, food manufacturers have been using stevia in the processing of many food stuffs for over 40 years (Chatsudthipong and Muanprasat, 2009; Thomas and Glade 2010). The main group of foods, in which stevia and its products have been used is quite surprising, namely salty products / food / snacks. It has

been reported that the combination of stevioside with sodium chloride gives a dish softness, and therefore its wide use in the production of pickled vegetables, dried seafood, soy sauces and miso products (Tadhani and Subhash, 2006; Goyal et al., 2010). Furthermore, sweet Stevia leaves are also utilized in beverages of reduced energy content, candies, chewing gums, baked goods, yogurts, ice creams, teas, and finally constitute a component of numerous toothpastes and mouthwashes. Of course, stevia is also used directly as a sweetener in the form of liquid, powder and tablets (Yadav et al., 2011; Lemus-Mondaca et al., 2012).

The Japanese are also pioneers in the purification of stevia extracts obtaining well-defined steviol glycosides characterized by an intensive sweet taste. Particularly noteworthy is rebaudioside A, because it has a more refined sweet taste without the characteristic bitter flavor symptomatic for other glycosides. Many methods for the extraction of stevia glycosides and innovative solutions in the application of sweetener mixtures, e.g. stevia with other natural and synthetic sweeteners, have been patented in Japan (Brahmachari et al., 2011; Yadav et al., 2011).

An important advantage of stevia is its stability during heat treatment, which facilitates its introduction into the recipes for many food products. Stevioside is stable at 95°C, and therefore it is suggested that the sweetener under investigation is suitable as a cooked food additive. At elevated temperatures and over one hour, the sweetener demonstrated good stability at a temperature up to 120°C, while at temperatures exceeding 140°C it underwent degradation, and its complete degradation occurs when exposed to 200°C (Kroyer, 2010). In addition, Kroyer found that aqueous solutions of steviol glycosides were degraded only at 5% at pH 2–10 (exposure for 2 hours, at 80°C). It has also been noted that stevia extracts are not fermented, and do not contribute to the browning reaction of cooked or baked products (Crammer and Ikan, 1986; Abou-Arab et al., 2010). Another advantage of stevia is that it does not form a precipitate in acid solution, which allows its use for the sweetening of carbonated beverages.

Currently, in the European Union since December 2011, steviol glycosides with the E 960 symbol have been approved for use in 31 food categories (Commission Regulation, 2011).

### **CONCLUSION**

Every year we observe increased interest in the application of stevia in food production. Stevia is a perfect answer to the needs of consumers, combining the qualities of a sweetener, but also constituting a source of many substances with a nutritional effect on the human organism. New types of food products enriched with stevia bring many benefits.

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### KIERUNKI WYKORZYSTANIA LIŚCI STEWII (STEVIA REBAUIDANA) JAKO DODATKÓW DO ŻYWNOŚCI

### **STRESZCZENIE**

Ogólnoświatowe trendy w technologii żywności legitymizują nadmierne wykorzystanie syntetycznych substancji słodzących w produktach spożywczych. Rozwój rynku żywności funkcjonalnej ukierunkowanej na wykorzystanie naturalnych dodatków pozwolił zwrócić uwagę na liście stewii (*Stevia rebauidana*), zawierające w składzie słodkie diterpeny glikozydowe. W pracy scharakteryzowano skład, wartość odżywczą oraz kierunki wykorzystania liści stewii.

Słowa kluczowe: stewia, Stevia rebaudiana, zdrowie, stewiozyd, rebaudiozyd, substancja słodząca

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