Stevia rebaudiana Bertoni - A Review of Nutritional and Biochemical Properties of Natural Sweetener

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Summary

Stevia as a natural sweetener with zero calories, and proven non-toxic effects on human health, recently found widespread use in the food and baking industry. Steviol glycosides that are concentrated in the plant leaves are: even 300 times sweeter than sucrose, heat-stable and well tolerate low pH-values. These are all characteristics that popularize this plant in its application as a natural sweetener in consummation and everyday use. This review provides an insight into: the possibilities of applying stevia as a sweetener for commercial purposes; biochemical and nutritional content of the stevia leaves; the basic techniques of its cultivation, and use of stevia leaves as a basic raw material for the various stevia products.

Key words

glycoside, stevioside, rebaudioside A, processing capabilities of stevia, phytochemicals

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Introduction

In general, human health is greatly endangered and characteristic diseases developed in the last decade due to excessive intake of "harmful" sugars present in food, beverages and in wide range of food products. From a medical point of view, increased use of products enriched with sugar (sucrose) favor the development of various chronic diseases. Obesity and diabetes are ones of the major diseases of modern mankind (Burke et al., 2003) and from the health aspect a great emphasis in the prevention thereof was placed. Increased interest of the consumer to reduce sugar intake through the food leads to higher popularization of products that instead of sucrose contain artificial sweeteners (50-100 times sweeter than sucrose) (Pól et al., 2007). Most artificial sweeteners with a high degree of sweetness are produced from synthetic ingredients or derived exclusively by chemical synthesis in the laboratory. Modern medical researches show that most artificial sweeteners are harmful for human health (Jae-Yong et al., 2011). Therefore, more and more sweeteners extracted from natural materials are used. Stevioside is natural sweetener isolated from the leaves of plant *Stevia rebaudiana* Bertoni and it is up to 300 times sweeter than sucrose (Alaam, 2007; Table 1). Dry leaves of stevia are sweeter approximately 10 to 15 times than sucrose (Raymond, 2010) while glycemic index is zero, so it is sweetener with no caloric value (Atteh et al., 2008; Kroyer, 2009; Seema, 2010; Puri et al., 2011) and with proven non-toxic effect on human health (Barriocanal et al., 2008).

Stevia rebaudiana Bertoni is native from northeastern Paraguay, and today it is cultivated around the world (Fig. 1).

The leaves of stevia naturally contain a mixture of 8 sweet diterpene glycosides: stevioside, steviolbioside, rebaudiosides (A, B, C, D and E) and dulcoside A (Geuns, 2003). Stevioside is present with an average of 4-20% in the dry matter of the plant leaves, which primarily depends on cultivar characteristics of plants and basic agricultural techniques (Brandle et al. 1992; Geuns 2000). From mentioned sweet glycosides enshrined in stevia leaves, in the highest percentage are present: stevioside, then rebaudioside A and other mentioned diterpene glycosides. Rebaudioside, regardles on smaller concentrations present in the stevia leaves comparing to stevioside, significantly contributes to a more pleasant sweet taste (Sahelin and Gates, 1999) than stevioside, which usually provides a somewhat bitter taste (Mitchell, 2006).

The basic raw material for the production of stevioside is the leaves of the stevia plant. Stems of plant contain very low concentrations of sweet glycosides and are removed during harvest to reduce the cost of further processing (Brandle et al., 1992). According to Jacobs (1973) during the harvest of leaves intended for the stevioside extraction, only green, healthy leaves are harvested from the stevia plant, while brown leaves and stems are removed. Specifically, green leaves contain a greater amount of chlorophyll A and B located in the chloroplasts of plant cells. The precursors of steviol glycosides are synthesized in chloroplasts so the tissues without chlorophyll pigment do not contain or contain only minor amounts of the sweet steviol glycosides (Brandle et al., 1992; Singh and Rao, 2005; Braz de Oliveira et al., 2011). Also, during the leaves drying process the structure of chlorophyll is changing and as a main consequence occurs the change in color from green to brown. This color change ultimately affects the change of color during the extraction and purification process of sweeteners (Abou-Arab et al., 2010). Sweetness i.e. stevioside concentration in the plant leaves is the highest just before flowering of plant, and the plants should be harvested as soon as flowering starts or before the first frost (Brandle et al., 1992).

Table 1. The relative sweetening strength of diterpene glycosides isolated from stevia leaves (Puri et al., 2011)

Diterpene glycosides	Relative sweetening strength
Stevioside	250-300
Rebaudioside A	350-450
Rebaudioside B	300-350
Rebaudioside C	50-120
Rebaudioside D	200-300
Rebaudioside E	250-300
Rebaudioside F	ND
Steviolbioside	100-125
Dulcoside A	50-120

Figure 1. Herbaceous shrub plant of Stevia rebaudiana Bertoni (photographed 2012)

The leaves of stevia are used as a food additive and a sweetener in food products. Usually, on the market they are present as: a green powder obtained by grinding of dried green leaves (Mishra et al., 2010), a white powder obtained by depigmentation process of green powder (Brandle et al., 1992) and a solution obtained by different extraction methods of stevioside and rebaudioside A from green powder (Abou-Arab et al., 2010).

Besides the high stevioside content, stevia plant is characterized as a good source of protein, dietary fiber, minerals and essential amino acids (Abou-Arab et al., 2010). Stevia leaf extract shows a high level of antioxidant activity, as well as the variety of phytochemicals such as phenolic compounds, which is directly associated with the removal of free electrons and superoxide radicals (Thomas and Glade, 2010; Benzie and Watchel-Galor, 2011). Therefore, stevia plant has significant potential for use as a natural antioxidant (Shukla et al., 2009; Ahmad et al., 2010; Kim et al., 2011).

Commercial use of stevia in Croatia and in the world

Popularization of stevia in food industry, and in general for commercial purposes, significantly started with its application in the production of world-popular nonalcoholic drink "Coca-Cola". With approval of FDA committee for consumption of stevia as a food supplement for sweetening, began its intensive cultivation and use of its products around the world and today it is commercially cultivated in Brazil, Uruguay, Central America, Israel, Thailand, Australia, Japan, Korea and China. Stevia largest producer is China with about 13 400 ha of planted area and about 40 000 tons of Stevia leaves every day. Also, China is the world's largest exporter of stevioside. As regards stevia cultivation in the countries of the European continent, there is still no accurate data of the planted area i.e. cultivation is still based on personal needs (Table 2).

Table 2. List of European countries where stevia is grown and researched (Sharma, 2007)

Country/ Location	Commercial production	Agricultural research	Non-agricultural research	Approved for use
Italy	–	+	+	–
United Kingdom	–	+	+	–
Ukraine/ Moldova	+	+	+	–
Spain	–	+	+	–
Germany	–	+	+	–
Sweden	–	–	+	–

"The International Stevia Council on November 14 (Brussels) has applauded the final approval of the European Commission's Regulation to authorize the use of steviol glycosides as a non-caloric sweetener in the European market. As a result, consumers across Europe will be able to enjoy products sweetened by steviol glycosides as early as December 2, 2011 (ingredient has been given the E number E960)" (www.preparedfoods.com).

In recent years, stevia products find widespread use in the food industry (Savita et al., 2004; Midmore and Rank, 2006). Steviol glycosides are used as a sweetener in many industrial foods, such as soft drinks or fruit juices (Goyal et al., 2010), desserts, sauces, delicacies, sweet corn, bread, biscuits and a table sweetener. Stevia diterpene replace sucrose in: cereals (muesli) (Wallin, 2007), pickles (Koyama et al., 2003), yoghurt (Amzad-Hossain et al., 2010), candy (Goyal et al., 2010), soybeans and soy sauce (Amzad-Hossain et al., 2010) and seafood (Goyal et al., 2010). The stevia leaves, as a food additive and a sweetener, on the market are present as a powder (green and white) and as a solution.

The preliminary research on the possibilities of stevia cultivation in Croatia started at the Department of Vegetable Crops, Faculty of Agriculture, University of Zagreb in 2009. According to valid legal regulations in Republic of Croatia, stevia is on the list of plants that can be used as a "dietary supplement" but not as an independent plant. In general, the possibility of legitimate use and sale of stevia products in Croatia is very limited i.e. sales and use of stevia leaves on the Croatian market is not allowed except in products with other plants.

Harvest and processing of the stevia leaves

Stevia leaves harvest can be single or multiple (leaves are harvested in specific intervals). The harvest usually starts in August and ends before the first frost. Sweetness i.e. stevioside content in stevia leaves is the highest just before flowering of plant, which is induced by short day length (Brandle et al., 1992). Beginning of flowering ranges from mid-summer to late fall (Fig. 2).

According to Novak yield of fresh and dry leaves per year ranged from 12 to 14 t ha^{-1} and 2 to 3 t ha^{-1} respectively. Only green and healthy stevia leaves are good raw material for further processing into various products.

After harvest, leaves should be air dried (natural drying) at lower temperature (40-50°C) during 24 to 48 hours (Mishra et al., 2010). The effect of dehydration process on stevioside level and nutritional quality of leaves is still not explored enough. Dehydration process of stevia leaves for longer than a day significantly reduces the stevioside content in the final product. Rajab et al. (2009) noted that drying at a temperature of 70-80°C for 8 hours significantly contributes to the preservation of the stevia leaves quality. Given that, the main requirement to be achieved in dehydration of stevia leaves is short and conducted period of drying at non-invasive

Figure 2. Stevia flowering (photographed, 2012)

(a) (b)

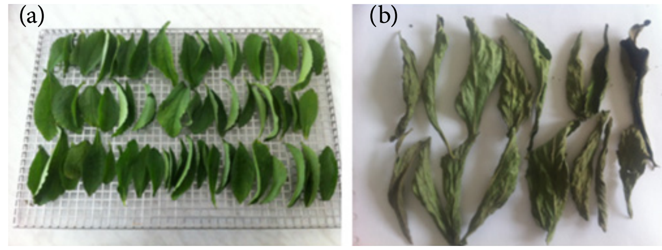

Figure 3. Stevia leaves before (a) and after dehydration process (b) in chamber drier (photographed, 2012)

temperatures. Preliminary research about stevia leaves dehydration was conducted in a laboratory drier using a temperature of 55°C with constant air flow for a period of 2 hours (Fig. 3) at Department of Agricultural Technology, Storage and Transport (Faculty of Agriculture, Zagreb).

Biochemical and nutritional aspects of stevia

Besides exceptional sweetness, stevia is popular due to nutritional and chemical composition that is characterized by content of amino acids, minerals and phytochemicals, especially polyphenols (Chu et al., 2000; Edeoga et al., 2005; Singh and Rao, 2012) that ultimately contributes to significant increase of antioxidant activity of plant (Chatsudthipong and Muanprasat, 2009; Ahmad et al., 2010). Opposite of the most commonly used sweeteners in the food processing (sucrose and artificial sweeteners), stevia stands out with health and nutritional values (Anton et al., 2010). According to the literature citations by other authors, it can be concluded that the dried stevia leaves are a good source of carbohydrates (35-62 g/100 g dry matter), proteins (10-20 g/100 g dry matter) and dietary fibers (15 to 18.5 g/100 g dry matter), which are very important in maintaining of human health (Abou-Arab et al., 2010; Mishra et al., 2010; Goyal et. al, 2012; Lemus-Mondaca et al., 2012). High ash content (6-13 g/100 g dry matter) indicates that stevia is a good source of minerals (potassium, calcium, sodium, magnesium, iron, etc). According to Abou-Arab et al. (2010) after extraction of stevioside, dried stevia leaves can be used as a valuable source of essential amino acids.

Diterpene glycosides of stevia

Stevia is a good source of phytochemicals, such as: austroinulin, β-carotene, dulcoside, nilacin, rebaudi oxide, riboflavin, steviol, stevioside and thiamine (Jayaraman et al., 2008). Natural sweetener isolated from the *Stevia rebaudiana* leaves are steviol glycosides, and according to chemical composition are diterpenes, isolated and identified as stevioside, steviolbioside, rebaudioside A, B, C, D, E, F and dulcoside (Geuns, 2003). In the stevia leaves, stevioside is the most common (4-13% w/w), and followed by rebaudioside (2-4% w/w), rebaudioside C (1-2% w/w) and dulcoside (0.4-0.7% w/w) (Makapugay et al., 1984; Gardana et al., 2010; Jackson et al., 2010; Cacciola et al., 2011). Diterpene glycosides were found in the highest percentage in the leaf of plant and make up 15% of the leaf chemical content which primarily depends on the cultivar (Giraldo et al., 2005). Diterpene content in stevia leaves depends on growing conditions (Pól et al., 2007) and the application of agricultural techniques (Genus, 2003) (Fig. 4). The content and distribution of sweet glycosides, primarily stevioside and rebaudioside, considerably varies depending on the part of the plant (root, stem or leaf). Chloroplasts are important precursors for the synthesis of stevioside and steviol glycosides. Plant tissues without chlorophyll (roots and lower part of stems) do not contain or contain only minor amounts of these glycosides. After flowering of plant, the level of glycoside starts to decrease (Pól et al., 2007).

There are three molecules of glucose and one molecule of steviol aglycone (diterpene carboxyl alcohol) in the stevioside chemical composition (Brandle and Telmer, 2007). Stevioside is even 300 times sweeter than sucrose with no caloric value and for that reason the stevia products are widely used as a sweetening agent

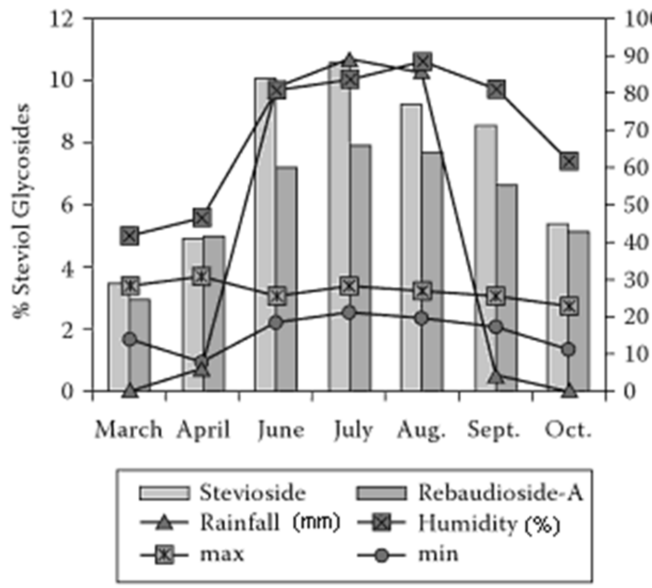

Figure 4. Effects of environmental conditions on the content of stevioside and rebaudioside A in stevia leaves during vegetation (Singh and Rao, 2005)

Table 3. Taste profiles of stevioside and rebaudioside A (Mitchell, 2006)

Compound	Taste quality (sweet/bitter/other)*
Stevioside	62/30/8
Rebaudioside A	85/12/3

* Percentage of the total taste sensation

primarily intended for people with diabetes (Puri et al., 2011; Nikolai et al., 2001). The flavors of the most abundant glycosides, stevioside and rebaudioside A, vary considerably (Kaushik et al., 2010). In general, stevioside gives the impression of slightly bitter taste, while rebaudioside A contributes to the typical sweet taste (similar to sucrose) (Singh and Rao, 2005; Table 3). Differences in taste are caused by more polar groups in rebaudioside A that enable the rebaudioside A better solubility and ultimately more similarity to taste of sucrose, unlike the molecule of stevioside. (Mitchell, 2006; Carakostas et al., 2008).

Both, stevioside and rebaudioside A are very stable molecules in aqueous solution in the wide range of pH and temperatures (Abou-Arab et al., 2010). Thus, during the thermal treatment (2 hours at 60°C) and pH in range 1 to 10, stevioside molecule indicates only a slight degradation, while significant loss (5%) was measured at a temperature of 80°C and pH values from 2 to 10 (Abou-Arab et al., 2010). Stevioside and rebaudioside A are thermally stable at higher temperatures, and have wide application in the food and in the baking industry (Tanaka, 1988). Abou-Arab et al. (2010) quoted termostability of stevioside even at temperature of 200°C, which allows a wide range of stevia commercial use (Fig. 5).

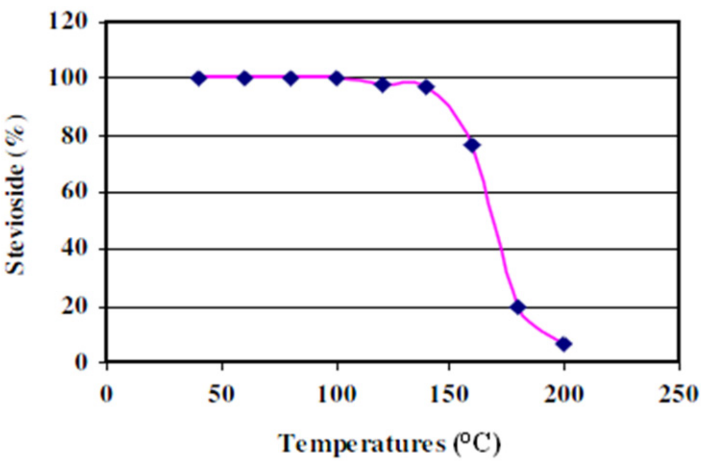

Figure 5. Stability and degradation rate of stevioside (50 mg) as a solid substance at high temperatures (40 - 200°C) for one hour (Abou-Arab et al., 2010)

Conclusion

Stevia rebaudiana Bertoni is an herb from South America that has found a great potential of use, primarily in the production of natural sweetener with emphasized sweetness. Due to its chemical structure and health-promoting phytochemical components, stevia is suitable for the extraction and production of functional food ingredients. Also, stevia is a good source of carbohydrates, proteins, dietary fibers, minerals, and amino acids. Stevia leaves contain diterpene glycosides that are low calorie sweeteners, with stevioside being there most abundant. Sweet diterpene glycosides are non-fermentative and non-toxic compounds that contribute to the flavor of a product or dish in which they are used. Diterpene glycosides do not have mutagenic, teratogenic, and carcinogenic properties.

Stevia has been consumed for centuries as a natural low calorie sweetener without any adverse effects on human health which is contrary to artificial sweeteners, which are determined by low caloric characteristics and a large percentage of carcinogenicity. Stevia for these reasons has a much greater advantage over other sweeteners (artificial sweeteners and sucrose) as an ingredient for the food industry and it is suitable as a replacement for sucrose in beverages, drinks and baked products. Besides the sweet content, owing to its secondary plant compounds (phytochemicals), stevia has antihyperglycemic, anti-inflammatory, anticancer, diuretic and immunomodulating effects.

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