Perspective

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A bioeconomy perspective for natural sweetener Stevia

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Abstract: The 'sweet herb' (*El Caa-ehe*), used by the Guaraní tribes living in the forests of today's eastern Paraguay and southern Brazil, is rapidly emerging as a natural sweetener that is an alternative both to sugar and synthetic sweeteners, well beyond Japan where it has been widely used since the mid-1970s. *Stevia rebaudiana* (Bertoni) Bertoni contains very sweet steviol glycosides in its leaves, which do not add calories and do not cause an increase in blood sugar levels. The glycoside that is most abundant in the leaves, stevioside, has high reactive oxygen species quenching activity and several properties that are beneficial to health. Rapid advances in green chemistry technology allow the production of stevia extracts that are devoid of any liquorice-like after taste. Their high chemical and physical stability enables them to be used in baked and beverage food products and this supports the large-scale use of stevia as a natural sweetener. Addressing bioeconomic aspects ranging from production to product formulation, this study identifies the last obstacles prior to general adoption of *S. rebadudiana* as a sweetener that is beneficial for health. © 2019 Society of Chemical Industry and John Wiley & Sons, Ltd

Keywords: *Stevia rebaudiana*; sweetener; bioeconomy; stevioside; rebaudioside; steviol

Introduction

Named by naturalist Moisés Santiago Bertoni after the botanist and physician, Pedro Jaime Esteve, who first described the *plant* found in eastern Paraguay in the mid-16th century, *Stevia rebaudiana* Bertoni (Bertoni) has rapidly become the main natural sweetener used across the world. The botanical name incorporates the name of Paraguayan chemist Ovidio Rebaudi, who conducted the first chemical analyses that aimed to clarify the origin of the sweet taste, upon the request of Bertoni in 1899.[1] In 2017, the number of new food and beverage products using stevia's extracts exceeded those using the synthetic sweetener aspartame.[2]

The plant's leaves contain glycosides of the diterpene steviol (*ent*-13-hydroxykaur- 16-en-19-oic acid) such as stevioside and rebaudiosides A, which are up to 400 times sweeter than sucrose (sugar), adding almost no calories in the body and not affecting blood sugar levels, thereby helping in calorie reduction. The glycosides do not undergo fermentation in the mouth and thus cause no tooth decay or cavity formation.[3]

Steviol glycosides were first successfully commercialized in Japan in 1971.[4] Their approval in the rest of the world was quite recent: they were approved in 2008 in the USA and in 2011 in the EU (as the high-purity stevia leaf extract labelled E 960). Today, stevia extracts are approved for use in food and beverage products in most of the

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world's countries, including large nations such as Russia, China, India, Canada, and Brazil.

In the European Union steviol glycosides have also been approved as dietary supplements with an acceptable daily intake of 4 mg of steviol (steviol glycosides) per kg of body weight[5] identical to the intake recommended by the WHO's Joint Experts Committee on Food Additives.

Following a burst of new studies in the late 1990s, which clarified its agricultural,[6] biological, and chemical properties, numerous recent studies have addressed the use of *Stevia rebaudiana* purified extract as a zero-calorie sweetener[7] and as a sweetener in pediatric oral formulations,[8] and have considered its future potential.[9]

We will not deal with the health and safety aspects of stevia because, as Talevi commented, 'an enormous body of preclinical evidence demonstrates the great pharmacological potential of *Stevia rebaudiana* (Bertoni) and its crude and purified extracts.'[3] Stevioside, for instance, actively counteracts high blood pressure, type 2 diabetes, arteriosclerosis and some forms of cancer, due to its excellent ability to scavenge reactive oxygen species, and hydroxyl and superoxide radicals in particular.[10]

The negative results in toxicity trials subsequently used by regulating authorities of other countries largely originate from tests carried out by Japanese scholars both on stevia extracts or single steviol glycosides during their evaluation of stevia as a possible sweetening agent in the 1970s.[11,12]

In general, it is well established nowadays that stevia extracts and high-purity steviol glycosides are safe to consume at or below the acceptable daily intake levels. Interested readers are referred to Geuns' 2012 account of safety evaluation of stevia and stevioside,[13] and to the recent comprehensive analysis by Talevi.[3]

This study offers a bioeconomy perspective on this prominent natural sweetener – one for which the bioeconomy – the use of renewable biological resources for industrial purposes – retains its instrumental environmental dimension.[14] International research projects financed with the aim of diversifying the cultivation of tobacco farmers with stevia,[15] new trade associations,[16] new international conferences,[17] and a global market boom[2] testify to the global uptake of steviol glycosides as the favored sweeteners of the near future.

In Europe, steviol glycosides are classified as food additives, and thus their use as food sweeteners (and not natural products) is regulated under European Parliament and Council Regulation (EC) No. 1333/2008 on food additives, and the only ingredients usable are steviol glycosides purified at 95%. The global demand for stevia is part of a megatrend (a global macroeconomic force originating in culture and society impacting business and the economy), which is driving the accelerated adoption of natural products that replace synthetic functional products in almost all segments of the food, cosmetics, and personal care industries.[18]

This study addresses bioeconomic aspects ranging from production to product formulation, and identifies the last obstacles to the general adoption of *S. rebadudiana* as a healthy alternative to synthetic sweeteners and to sugar.

Composition, analysis and extraction routes

The main steviol glycosides responsible for the sweet taste of the *Stevia rebaudiana* leaves are stevioside (5–10% w/w on a dry weight basis), rebaudiosides A (2–5%) and C (1%), dulcoside A (0.5%), rebaudiosides D, E, and F (0.2%), and steviolbioside (0.1%).[18] The overall number of *ent*-kaurane diterpenoid glycosides identified in trace amounts was 34 as of April 2013,[19] and now exceeds the 40 threshold. These include rebaudioside M, a compound with a clean, sweet taste, and moderate licorice aftertaste abundant in a *S. rebaudiana* plant cultivar resulting from a controlled breeding program or from recombinant microorganisms, which was approved by the European Food Safety Authority (EFSA) Panel on Food Additives and Nutrient Sources added to Food.[20]

Stevioside was first isolated in France by Bridel and Lavieille in 1931,[21] and the structure and stereochemistry of the steviol aglycon, and the highly stable isosteviol formed by acid treatment of steviol via the Wagner–Meerwein rearrangement, were identified in 1960.[22]

The content of the two major steviol glycosides, stevioside (ST, Fig. 1) and rebaudioside A (Reb A, Fig. 1), is measured using high-performance liquid chromatography (HPLC) according to a standard procedure recommended by the Joint FAO WHO Expert Committee on Food Additives in 2010.

The method typically requires 25 min to determine the weight percentage of seven steviol glycosides (stevioside, rubusoside, dulcoside A, steviolbioside, rebaudioside C, rebaudioside B, and rebaudioside A) by UV detection at 210 nm using 80/20 acetonitrile/water at pH = 3.0, adjusted with phosphoric acid.[23]

More recently, Zimmermann in Germany has achieved the separation of nine steviol glycosides using a faster ultra-high performance liquid chromatography (UHPLC) method, reducing to 11 min the overall separation time

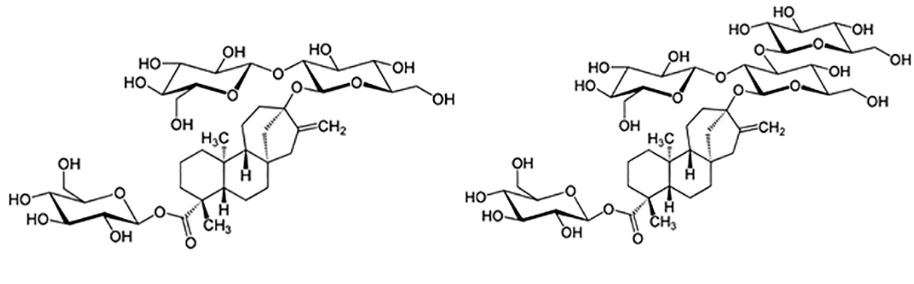

Figure 1. Stevioside and rebaudioside A, molecular structures.

for nine steviol glycosides, proposing that the method undergo multi-laboratory validation to prove its suitability as the new official method.[24]

Similarly, McChesney and co-workers have recently developed a chromatographic method using acetonitrile : water to separate and analyze fractions rich in very polar steviol glycosides.[25] In general, the higher the Reb A and ST glycoside content in the dried leaves, the higher is the price paid to farmers by the stevia processing companies. The optimum harvest date coincides with the bud-flowering stage at the beginning of September.[26]

Numerous agricultural efforts aim to improve steviol glycosides in leaf tissues, preferably avoiding gene manipulation as consumers looking for natural products are interested in GMO-free stevia. From microwave-assisted extraction to rapid solid-liquid dynamic extraction, several new technologies for the recovery of phytochemicals from stevia's leaves have been investigated.[27]

Recently, scholars in Brazil demonstrated how a simple pre-treatment of the leaves of *S. rebaudiana* with a high content of rebaudioside A with absolute ethanol increases the yield and purity level of stevia sweetener, also significantly enhancing the sensory characteristics of the steviol glycoside extract.[28] In brief, the treatment of stevia leaves with ethanol before the extraction of steviol glycosides selectively removes substances, such as phenolic compounds and flavonoids, which contribute to the residual bitter taste in the final product.

The aqueous extraction of steviol glycosides (Fig. 2) was optimized via response surface methodology. Eventually, the *S. rebaudiana* leaves were extracted with water (1:27, w/v at 60 °C and 200 rpm in three cycles). The crude extract was then filtered under a vacuum to remove the suspended particles. For purification, the filtered crude extract passed through UF (10 kDa) and NF (500 Da) membranes with an ion exchange and adsorption column eluting with ethanol : water (85:15, v/v). The purified sweetener in powder form was obtained via simple rotary evaporation.

An increase of 43% in yield of steviol glycosides was obtained from treated leaves (recovery went from 2.20 g for in natural leaves to 4.02 g for pretreated leaves), with levels of purity of 87% and 84.8%, respectively. Remarkably, from the point of view of the final use of the extract as natural sweetener, the scholars found that the antioxidant activity, phenolic compounds and flavonoids *increased*, whereas arachidic and beenic fatty acids were detected only in the leaves treated with EtOH. Furthermore, sensorial evaluation demonstrated that the extract from pretreated leaves presented a sensory profile similar to that of the synthetic sweetener sucralose, a chlorinated sugar, with the acceptance of the sweetener consumed by the treated leaves (7.00 ± 1.95) even higher than that of the world's most used synthetic sweetener (6.33 ± 1.72).[28]

The sweetener obtained was 165 times sweeter than sucrose, and the bitterness threshold was 0.073 ± 0.013 g/100 mL, considerably higher than the threshold of bitterness of stevioside (0.0172 ± 0.0050 g/100 mL) and enzymatically modified stevioside (0.0263 ± 0.0056 g/100 mL), indicating that it was significantly less bitter.

On the other hand, the ethanolic extract included several compounds that present excellent potential for use in foods, beverages, nutraceuticals, cosmetics, and personal care products. Indeed, the ethanolic extract showed high antioxidant potential and 39 compounds were identified by ultra-performance liquid chromatography coupled to high-resolution mass spectrometry (UPLC/HRMS).

Mass spectrometry investigation of the stevia extracts shows that centaureidin, epigallocatechin gallate, coumaric, caffeic, coumaroylquinic, and chlorogenic acids are the most abundant phenol antioxidants. These compounds include flavonoid derivatives (quercetin, centaureidin, epigallocatechin gallate, luteolin-glucoside), and hydroxycinnamic acids (coumaric, caffeic, chlorogenic, and coumaroylquinic acids).[29]

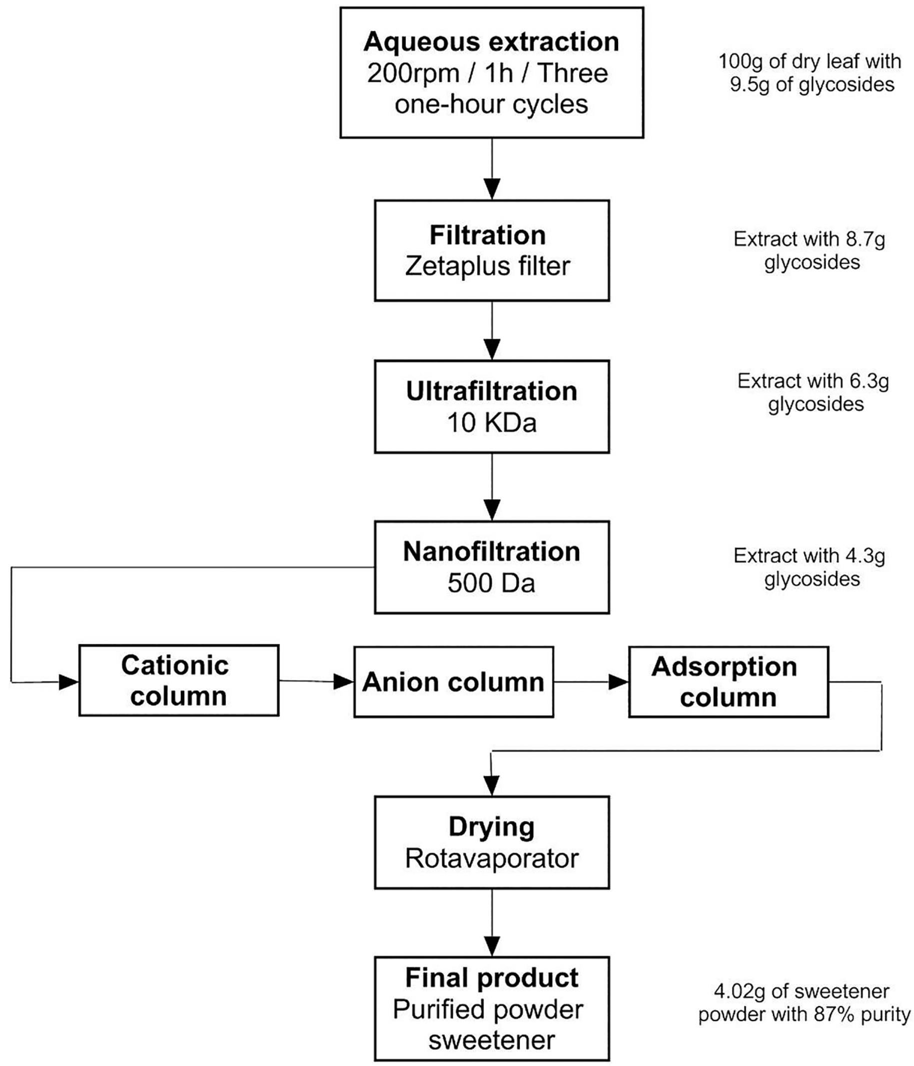

Figure 2. Flowchart for obtaining the enhanced sweetener powder from the leaves of *S. Rebaudiana* after preliminary treatment with absolute ethanol. [Reproduced from Ref. 28, with kind permission].

These extracts, rich in flavonoids and hydroxycinnamic acids, have strong reducing properties. For example, they readily reduce Ag^+ in solution affording silver nanoparticles of spherical shape with 16–25 nm average sizes in a truly green synthesis of metal nanoparticles, which is of great practical relevance in today's industrial context in which metal nanoparticles find a large and ever increasing number of applications.[29]

$$R{-}OH \rightarrow R{-}O^- + H^+ \tag{1}$$

$$R{-}O^- \rightarrow R{-}O^\bullet + e^- \tag{2}$$

Electrons are made available via the dissociation of hydroxyl groups of phenol compounds (Eqn (1)) and the subsequent detachment of electrons from $R{-}O^-$ anions (Eqn (2)), as the energy of electron transfer from deprotonated molecules is significantly lower than that required for hydrogen or electron detachment from the whole molecule.[29]

Uses, market, and perspective

Sweeteners from stevia extracts, available on the marketplace in powder, tablet, and liquid form, are widely used today to sweeten a variety of beverages and food products.[7] Indeed, steviol glycosides display remarkable physical and chemical stability, allowing their use in acidic beverages including cooked foodstuffs, biscuits, bakery goods, dressings, sauces, frozen foods, processed fruits and vegetables, snacks, and cereals. In personal-care products, stevia-based extracts are used in toothpaste and mouthwash formulations, replacing saccharin and older synthetic sweeteners and offering further purifying action.

As mentioned above, innovation efforts in agriculture have led to optimal varietal selection with the objective of selecting plants with the best organoleptic result, preferably without the use of enzymes or genetically modified plants. Variants of the stevia plant producing more glycosides than others are selected as cultivars, further optimizing the cultivation conditions to grow the plant optimally in different climates.

Forecasts, dating back to 2013, predicting that stevia would be 'unlikely to widely replace other high intensity sweeteners in the medium term'[30] turned out to be inaccurate. In 2017 the number of new commercial products sweetened with steviol glycosides outpaced those using aspartame.[2]

As with pectin, another natural product in great demand,[31] figures concerning market size and estimates about its growth differ greatly. According to one reputed market intelligence company, the global stevia market generated $338 million in 2015 and was anticipated to grow to $554 million by 2024 (at 6.1% compound annual growth rate).[32]

According to another analyst, the global stevia market generated $417 million in revenues in 2017 and was forecast to value $721 million by the end of 2024, growing at an 8.2% annual rate.[33] Finally, another market intelligence company estimates that, based on current trends, which saw the market launch over 14 000 food and beverage products with stevia across the world between 2011 and 2016,[34] the global market should exceed $1 billion by 2021.[35]

Currently, *S. rebaudiana* is mainly cultivated in China, Japan, Malaysia, Indonesia, Thailand, and Armenia, in Asia, and in Kenya, Ghana, Rwanda, Morocco, Zambia, Tanzania, and Congo in Africa. Cultivation in large and sunny countries such as India, Turkey, Mexico and the USA, is currently being expanded rapidly.

In Turkey, for example, by mid-2017 dried stevia leaves obtained from cultivation of the plant in a wet area where it is harvested twice a year, were sold at $150/kg.[36] In that farm 1 ha affords 123 kg of the end product, so revenues for the farming company in that area of Turkey were exceeding $18 400 per ha.

It is instructive to review the market prices in the last decade, namely the first of the stevia era as a global sweetener. In the USA, on the basis of the favorable review by the Joint FAO WHO Expert Committee on Food Additives in Europe,[37] several food and beverage products by different manufacturers were granted the 'generally recognized as safe' (GRAS) status,[38,7] and the production of stevia purified extracts quickly went from 5000 tonnes in 2008 to over 20 000 tonnes in 2011.[39] This caused overcapacity as demand was growing at a slower pace.

In 2013 the prices of Reb A 95% pure (Reb A 95) in China started to decline from over $110 000/t to less than $99 000/t by early 2014, recovering since the beginning of 2014, due to the scaling down of production by Chinese suppliers. This led a reputed market analyst to argue that 'the price of stevia was already high compared to other sweeteners, and these new increases could be limiting the expansion of the industry.'[39]

However, in the subsequent three years the continuing growth of new products containing *S. Rebaudiana* extracts further boosted production, and the price of 1 t of Reb A 95 went from $122 000 in January 2015 to to $73 000 in July of that year.[39] Almost two years later, by February 2017, prices of Reb A 95 were almost unchanged, at $77 300 per tonne.[40]

Three years before, a thorough market study conducted in Malaysia, which aimed to investigate the factors that influence the acceptance of stevia-based products by consumers, had revealed that most of the respondents were willing to use stevia-based products as a substitute for sugar.[41] Consumers with higher levels of education showed more willingness to change for stevia-based products because of their health benefits, leading the team to conclude that effective promotion was necessary to increase consumers' awareness of the need for a healthier diet.[41]

Conclusions and recommendations

In conclusion, this study has identified the last obstacles to the general adoption of *Stevia rebaudiana* Bertoni (Bertoni) as a sweetener that is beneficial to health. They are summarized below.

First, the use of stevia as natural sweetener as alternative to artificial sweeteners should focus on the consumer's desire to achieve outcomes such as better health, lower weight, and slower aging. In the safety review of stevia that accompanied a GRAS affirmation petition in 1992, Douglas Kinghorn wrote: 'the vast majority of the scientific safety evaluation studies which have been performed to date endorse the use of *Stevia rebaudiana* leaf and stevioside as sucrose substitutes. This is substantiated by the extensive use in Japan of these products without a single adverse report to date.'[42]

Since then, the use of *S. rebaudiana* as sweetener has extended to almost all countries with many new health beneficial effects having been discovered. Remarking how 'consumers can be provided with the best nutritional knowledge and palatable well-balanced food options and still make choices that are not good for their physical health' de la Peña has noted how, in the history of artificial sweeteners adoption in the USA, 'the many failures of nutrition education in the 20th century suggest that we are missing something important in how we think about the relationship between consumer desire and healthy food consumption . . . as these foods promise to deliver more than fullness. They promise that through their ingestion we will become more of what we desire to be.[43]

Second, companies trying to promote the adoption of stevia as a universal sweetener will benefit from learning the approach chosen by a consortium of four companies in France, including Europe's leading manufacturer of purified steviol glycosides.[44] Aware that industrial customers have been accustomed to formulating food and beverage products with artificial sweeteners for several decades, the consortium offers prospective customers the unique expertise of one of its partnering companies to provide guidance on *how* to do this with steviol glycosides.

Third, stevia's stakeholders and companies may benefit from learning what the Stevia Konwakai (today renamed Stevia Kogyokai), Japan's stevia industrial consortium, has done after the ban on dulcin and cyclamate in Japan in 1969, to promote the uptake of stevia sweeteners (first mixed with licorice-derived glycyrrhizin and later on with 'rebaudioside A-enriched stevia extract').[45] This commercial success was achieved not only through collaborative work in obtaining permission for the use of, and registration of, stevia as a sucrose substitute, but also through the exchange of information on production, marketing, and research.[45]

Fourth, noting that misconceptions surrounding stevia continue to slow progress in its uptake, delaying an opportunity to minimize the incidence of diabetes and obesity,[46] practitioners of the bioeconomy should be aware that progress in formulating sweeteners based on steviol glycosides has been so significant that today's children were found to prefer skim chocolate sweetened with a commercial stevia sweetener white powder soluble in water produced from aqueous extract of stevia leaves (95% on a dry weight basis in steviol glycosides, rebaudioside A plus stevioside >75%).[47] As shown recently by Formigoni's research team in Brazil,[28] simple treatment of the stevia leaves with absolute ethanol before the aqueous extraction of steviol glycosides selectively removes the phenolic compounds and flavonoids that contribute to the residual bitter taste in the final product, resulting in steviol glycosides whose sensory profile is similar to (and better than) that of sucralose.

Fifth, when substituted for sugar, stevia can be used by anyone, including people of normal weight who are aiming to reduce their overall sugar intake and improve the quality of their diet.[48] Hence, forward-looking companies may directly avoid using stevia in combination with sugar or with artificial sweeteners but rather formulate natural stevia's extracts with natural bulking and hydrocolloid agents such as pectin or natural dietary fiber.[49]

Eventually, due to rising concerns about the role of sugar consumption in the global epidemics of obesity and type 2 diabetes,[50] stevia will compete not only with artificial high-intensity sweeteners but also directly with sugar (sucrose) and high-fructose corn syrup.

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