Review

Main properties of steviol glycosides and their potential in the food industry: a review

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Received 7 July 2013
Accepted 10 September 2013

Fruits, 2014, vol. 69, p. 127–141

DOI: 10.1051/fruits/2014003
www.fruits-journal.org

RESUMEN ESPAÑOL, *p. 141*

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Abstract – Introduction. In recent decades, there has been increased interest in developing natural non-caloric sweeteners, providing sensory properties similar to sucrose. One of the most promising alternatives is the steviol glycosides, high-sweetness compounds extracted from the leaves of *Stevia rebaudiana* Bertoni. **Characteristics and properties**. In this review article, the characteristics of this plant are described. According to the relevant literature data, this review provides insight into the major steviol glycosides as well as their main characteristics and properties. **Toxicity and legislation**. We describe the current situation on the toxicity and the legal framework of the above sweeteners. **Extraction techniques**. This review describes the techniques developed for extraction and purification, including conventional extraction with water/solvent, ion exchange, enzymatic extraction, supercritical fluid and with a focus on membrane filtration, for its proven advantages in the purification of aqueous extract obtained by leaching of the leaves. **Applications**. This review also presents various evidence relating to the implementation of steviol glycosides in the food industry, with an emphasis on their use in the formulation of fruit juices. Conclusions. Finally, the future prospects of these natural sweeteners are presented. They highlight the need for research in this area, and the great appeal and commercial potential of such compounds, which should be focused on continuous assessment in relation to issues such as the intensity, persistence of sweetness and the absence of other residual flavors, in order to ensure their acceptance, preference and choice by the general public.

***Stevia rebaudiana* / leaves / extraction / glycosides / sweeteners / stevioside / food industry / fruit juices**

Synthèse sur les principales caractéristiques des glycosides de stéviol et leur intérêt dans l'industrie alimentaire.

Résumé – Introduction. Au cours des dernières décennies, il est apparu un intérêt croissant pour le développement des édulcorants naturels, non-caloriques, offrant des propriétés sensorielles similaires à celles du saccharose. Parmi les produits les plus prometteurs apparaissent les glycosides de stéviol, composés très édulcorants extraits des feuilles de *Stevia rebaudiana* Bertoni. **Caractéristiques et propriétés**. Dans cette synthèse, nous décrivons les caractéristiques de *S. rebaudiana*. À partir des données de la littérature, cette étude donne un aperçu des principaux glycosides de stéviol et de leurs principales caractéristiques et propriétés ; elle souligne d'autres qualités de ces produits. **Toxicité et législation**. Nous faisons un point sur la toxicité et le cadre juridique des édulcorants concernés. **Techniques d'extraction**. Notre synthèse décrit les techniques mises au point pour l'extraction et la purification, y compris l'extraction conventionnelle avec de l'eau ou solvant, l'échange d'ions, l'extraction enzymatique et le fluide supercritique ; elle met l'accent sur la filtration membranaire du fait de ses avantages prouvés dans la purification de l'extrait aqueux obtenu par lixiviation des feuilles de *Stevia*. **Applications**. Notre synthèse présente également divers éléments relatifs à la mise en œuvre des glycosides de stéviol dans l'industrie alimentaire, en insistant sur leur utilisation dans l'élaboration de jus de fruits. **Conclusions**. Enfin, basées sur le jugement des auteurs, les perspectives d'avenir de ces édulcorants naturels sont présentées. Elles soulignent le besoin de recherches dans ce domaine, le grand attrait et le potentiel commercial de ces composés. Des recherches devraient être axées sur l'évaluation de caractéristiques telles que l'intensité et la persistance du goût édulcorant, ainsi que l'absence d'autres saveurs résiduelles, aptes à assurer l'acceptation de ces produits, leur préférence et leur choix par le grand public.

***Stevia rebaudiana* / feuille / extraction / glycoside / édulcorant / stévioside / industrie alimentaire / jus de fruits**

1. Introduction

Currently, the sweeteners used in the formulation of low-calorie foods, such as acesulfame-K, aspartame, neotame, saccharin and sucralose, are high-intensity synthetic compounds. As well as being innutritious, many of these sweeteners have been shown to be carcinogenic and are associated with various other diseases [1]. For this reason the food industry, in recent years, has dedicated considerable effort to the development of alternative sweetening agents with similar characteristics and properties, but obtained from natural sources. One of the most important features that have been sought in these natural alternatives is the similarity of their sensory profile to that of sucrose and other ingredients, in order to provide a high-quality product which also generates a wide sensory acceptance [2].

A small number of low-calorie natural compounds, such as, for example, thaumatin, glycyrrhizin, xylitol, phyllodulcin, mogroside and stevioside, are now used commercially in several products [3]. Of these, the compounds obtained from *Stevia rebaudiana*, principally stevioside and rebaudioside A, have, in the last decade, generated considerable interest, due to their high solubility in water, intense sweetness and synergistic effects with other sweeteners. Stevioside and rebaudioside A are extracted from the leaves of *S. rebaudiana* Bertoni; they are available commercially in Japan, Korea, China, South-East Asia and South America, where they have been used for several decades to sweeten a variety of foods, including drinks, sweet vegetables, pickles and seafood [4].

On the other hand, and particularly in beverages and fruit juices, the sensory properties and organoleptic attributes of the final product are influenced by several factors such as the type and quantity of acids and other added sweeteners. In the present review, we describe the latest developments that have taken place as regards the properties, some extraction techniques and analysis of the steviol glycosides present in the leaves of *S. rebaudiana*. We then discuss, with specific examples, their application in the formulation of foods based on fruits.

2. Main characteristics of steviol glycosides

Stevia rebaudiana Bertoni is a herbaceous plant in the Asteraceae (Compositae), native to Paraguay, and mainly found in certain regions of South America [5].

The commercial exploitation of Stevia has risen significantly since the 1970s, when several Japanese researchers developed a series of protocols for the extraction and refining of the sweetener compounds, diterpene glycosides, found in its leaves [6]. More than 30 of these compounds have been reported to date [7, 8]; among them, the best known molecules are stevioside and rebaudioside A (*figure 1*, *table I*). Stevioside is normally found in greater quantities than the other compounds, between 4% and 20% weight of the dried leaves, and it is known that it is about 300 times sweeter than sucrose [5]. Stevia sweetness mainly depends on the genetic characteristics (variety), although several successful crop agronomic improvements have recently been implemented to raise the proportion of rebaudioside A; the variety Morita II has gained more relevance, presenting this quality [5].

As a result of the dominance of stevioside, the crude extracts obtained from the leaching of Stevia leaves have a characteristic bitter aftertaste [6]. Nevertheless, extracts with higher proportions of rebaudioside A have a much improved flavor profile, due to their physico-chemical and organoleptic characteristics. Rebaudioside A is also highly soluble in water, thus facilitating its use in food elaboration [6].

Stevioside and rebaudioside A have multiple advantages as dietary supplements. They are non-metabolizable (non-caloric), non-fermentable and do not cause dental caries. Another notable benefit is that the consumption of both steviol glycosides is safe for human health and there are no restrictions on their use by people suffering from diabetes [9, 10]. Stevioside and rebaudioside A represent the few natural sweeteners that are non-caloric, ready-made in nature and that can be reliably produced at an industrial level. In fact, high doses of stevioside (750–1500 mg per day) have been

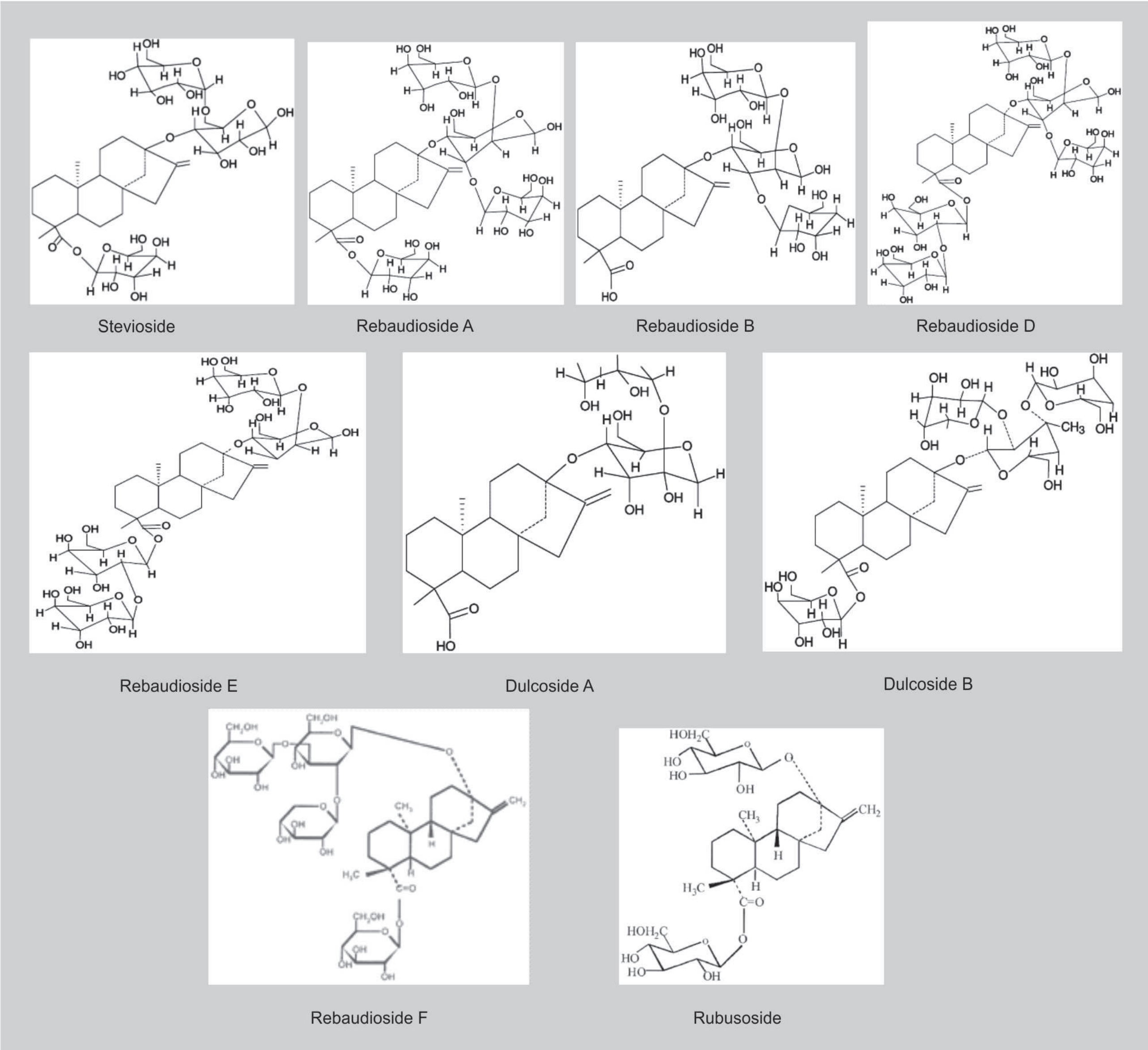

Figure 1.
Chemical structure of major glycosides found in *Stevia rebaudiana* Bertoni [3, 42].

used with favorable results for the treatment of hypertension and type 2 diabetes [13, 14].

The therapeutic value of stevioside consists of the fact that it substitutes sugar whilst at the same time stimulating the secretion of insulin in the pancreas during the treatment of diabetes and other carbohydrate metabolism disorders [9, 15].

Thus, obese people are able to lose weight, thanks to the fact that they can continue to eat sweet foods whilst lowering their sugar intake [16]. In addition, steviol glycosides have been shown to participate in the reduction of blood cholesterol [16] and improve cell regeneration and blood clotting, as well as suppressing neoplastic growth and strengthening blood vessels [17, 18].

Stevioside and rebaudioside A also exhibit anti-inflammatory and diuretic properties [19], prevent ulceration of the gastrointestinal tract [18, 20] and show anti-hyperglycemic [21–23], anti-hypertensive,

Table I.
Glycosides found in *Stevia rebaudiana* leaves [8, 11, 12].

Glycoside	Molecular formula	Sweetening power (with reference to saccharose)	Concentration in leaf (% w/w)	Proportion of glycoside in the leaves (% of total steviol glycosides)
Stevioside	$C_{38}H_{60}O_{18}$	150–300	4–14	43.1–79.6
Rebaudioside A	$C_{44}H_{70}O_{23}$	250–450	2–4	1.6–9.9
Rebaudioside C	$C_{44}H_{70}O_{22}$	120–500	1–2	0.5–6.0
Steviolbioside	$C_{32}H_{50}O_{13}$	100–125	< 0.4	0.3–3
Rubusoside	$C_{32}H_{50}O_{13}$	100–120	< 0.4	Undefined
Rebaudioside B	$C_{38}H_{60}O_{18}$	300–350	< 0.4	0–0.02
Rebaudioside D	$C_{50}H_{80}O_{28}$	250–450	< 0.4	0–0.4
Rebaudioside E	$C_{44}H_{70}O_{23}$	150–300	< 0.4	5.5–43.2
Rebaudioside F	$C_{43}H_{68}O_{22}$	Undefined	< 0.4	0.04–0.1
Dulcoside A	$C_{38}H_{60}O_{17}$	50–120	0.4–0.7	0.2–0.4

anti-tumor, anti-diarrheal and immunomodulatory activities [24]. The above-mentioned beneficial effects of steviol glycosides prove that they could be useful not only as natural sweeteners, but also as agents of preventive chemotherapy [15]. Recently, further properties of *Stevia* leaf extracts have been reported, including antibacterial, antiamnesic and antiviral activities. They have also been shown to have positive therapeutic effects in the treatment of neuralgia, anemia, lumbago, rheumatism, eczema and dermatitis [25].

3. Toxicity and legislation

One of the strongest arguments for the safety of Stevia for human health is that there have been no reported adverse effects over more than hundreds of years of continuous use by Paraguayans [3]. A comprehensive examination of the toxicity of stevioside and related compounds, emphasizing their therapeutic benefits, has been reviewed [26–28]. In addition, safety assessments of stevioside and the corresponding clinical trials have been well documented in a recent article [28]. These and other reports show that daily oral consumption at a reasonable level of stevioside (5 $mg \cdot kg^{-1}$ of body weight) is safe and is also guaranteed to be neither carcinogenic nor mutagenic [15].

All these studies provide clear evidence that the extracts obtained from *Stevia* leaves can be safely used by both healthy people and diabetics, as well as providing an important source of antioxidants in the human diet [15].

With regard to finished product marketing regulations drawn up for Stevia-based sweeteners in the United States, in recent years the Food and Drug Administration (FDA) has granted the respective permissions for their use in food to companies such as Cargill and Merisant. In Europe, Switzerland, as well as a large number of countries, currently allows the use of Stevia sweeteners in foods and beverages, but the procedure required for the commercialization and approval of the finished products is cumbersome. On the other hand, France approved Stevia-based products in 2010 [3].

4. Stability of steviol glycosides

Several investigations have demonstrated the stability of stevioside and rebaudioside A under room temperature, neutral pH and sunlight conditions. These sweeteners can be used under pH conditions ranging from 2 to 10 and at temperatures of up to 120 °C [29–34].

The stability of a stevioside-based sweetener in solutions formulated with different organic acids (citric, tartaric and phosphoric acid) and vitamins (thiamine, riboflavin, niacin and pyridoxine) under different storage conditions was evaluated [29]. Its application as a sweetener in coffee and tea was also assessed. The stevioside showed stability when incubated at a high temperature (120 °C) for one hour, although it degraded at temperatures above 140 °C [30]. An aqueous solution of the stevioside remained stable over a pH range of 2 to 10 at 80 °C. Stevioside content did not exhibit significant changes when incubated for up to 4 h with water-soluble vitamins at 80 °C. When subjected to extremely acid conditions (pH 1), however, a significant decrease in stevioside concentration was observed. As expected, the authors found that ascorbic acid had a protective effect against the degradation of the stevioside. No interaction at room temperature was found after 4 months of incubation of the aqueous media. Studies on the stability of the stevioside in organic acid solutions showed a tendency to greater decomposition of the sweetener at low pH, depending on the acid media.

Moreover, the stability of steviol glycosides in diverse food products [semi-skimmed milk, soy drinks, fermented milk drinks, ice cream, yogurt (made with both full cream and skimmed milk), dry biscuits and jam] was assessed [31]. Storage conditions were those recommended for each type of food (–18 °C for ice cream, 6 °C for yogurt and 20 °C for skimmed and fermented milk). Stevioside recovery was between 96% and 103%, thus demonstrating that the steviol glycosides had not decomposed in any of the samples tested.

Meanwhile, Praskash *et al.* evaluated the stability of stevioside and rebaudioside A and the possible formation of steviol aglycone in different soft drinks [32]. Samples made with these sweeteners were stored for (24, 48 and 72) h at 80 °C before analysis. The steviosides degraded by over 70%, with rebaudioside A showing greater stability than stevioside. The highest level of degradation (71%) was observed in lemonade with caffeine after 72 h, while the energy drink degraded by just 27% (the value referred to degradation of stevioside). They also reported, as for previous publications [33], that the *in vitro* synthesis of stevioside and rebaudioside A, after incubation with human microflora under strict anaerobic conditions, produced a degradation of stevioside to steviol after 10 h, whereas rebaudioside A was completely degraded after 24 h. *In vitro* tests with rat intestinal microflora showed a complete degradation of stevioside in 2 days, while that of rebaudioside A required 6 days.

Finally, the stability of rebaudioside A in solutions at a pH range of 2.8–4.2 (simulating normal conditions of commercial beverages) was studied [34]. Specifically, solutions with a pH between 2.8 and 3.2 were used to simulate lemon-lime-flavored drinks, solutions with a pH of 3.8 for soft drinks and pH 4.2 for root beer. Samples were stored at different temperatures [(5, 20, 30, and 40) °C] for 26 weeks. The results indicated that 7 to 12 minor degradation products were obtained from rebaudioside A without appreciable loss of sweetness. The stability of rebaudioside A, which depends on pH, temperature and storage time, was also studied. The degree of degradation increases with lower pH, and higher temperature and storage time.

5. Techniques for the extraction and purification of steviol glycosides

Several studies have focused on the evaluation of different methods and techniques for the extraction of steviol glycosides from *Stevia* leaves.

The most used is the conventional extraction method (extraction medium: hot water) [5] where powdered *Stevia* leaves were mixed with hot distilled water [(78 ± 1) °C] at a 1:14 (g:mL) ratio in a water bath for 56 min. The aqueous extract was then cooled to room temperature and filtered before centrifuging at an agitation speed of 5334 *g* for 26 min in order to obtain a primary clarification of the extract. This research also evaluated the use of fresh *Stevia* leaves for the preparation of aqueous Stevia solution at a concentration of 10%. The sweeteners from dry *Stevia* leaves were extracted in cold water for 2 h. The solids were then separated from the solution by vacuum filtration [5].

Similarly, Vanneste *et al.* used cold water as a solvent to avoid extracting larger impurities which are dragged along by heat and are then much more difficult to remove using membranes [13]. A solution of 20 g of leaves per liter of water was prepared and shaken for 2 h at 5 °C. This method gave a yield of 11% steviol glycosides: 7% stevioside and 4% rebaudioside A. The extract was then passed through a 63-μm mesh to eliminate the remaining material, thus avoiding fouling of the filtration equipment.

Another popular extraction solvent for conventional extraction of Stevia glycosides is alcohol, specifically ethanol [15]. In it, an aliquot of 100 g of *S. rebaudiana* leaves were mixed with 1000 mL of ethanol and water, and extracted using a Soxhlet (500 mL) run for two cycles (approximately 100 min). The extract was then concentrated until dryness at 70 °C in a rotary evaporator under vacuum and then lyophilized. Similarly, a quantity of 5 kg dry *S. rebaudiana* leaves was treated and soaked in hexane to eliminate undesired pigments and other waxy material present on the surface of the *Stevia* leaves [28]. The dry leaves were then crushed to a fine powder. The retrieved leaf powder was then soaked in a pH 5 aqueous solution at a ratio of 1:10 (leaf: water) and shaken for 2–3 h at 80 °C. After shaking, the solution was extracted with pressurized *hot water extraction* (PHWE) for 10 min under the following conditions: 10 kPa pressure, 120 rpm, temperature 100–110 °C. This protocol could be considered as a pre-treatment for the clarification of the crude extract. Optimum extraction conditions were revealed as 211 bar, 80 °C and 17.4%, which yielded 36.66 mg·g^{-1} stevioside and 17.79 mg·g^{-1} rebaudioside A. The total glycoside composition was close to those obtained using conventional water extraction (64.49 mg·g^{-1}) and a little higher than ethanol extraction (48.60 mg·g^{-1}).

Also, Ion Exchange Extraction was tested [35]. The authors describe a method of extraction and clarification by selective absorption with X and A zeolites. They indicate the efficiency of barium and calcium ions in NaX and NaA zeolites during the ion exchange phase, and demonstrate that the use of the CaX zeolite during extraction improves clarification without producing alterations in the initial characteristics of concentration, glycosides and flavor. The results indicate that one may obtain in the first 2 h (according to the proposed conditions) a high clarification percentage, followed by a subsequent gradual decline. With a flow rate of 1.25 mL·min^{-1}, clarification becomes approximately constant during 90% of the time with a clarification of between 55–60% in the whole process. Lastly, the authors highlight the possibility of zeolite reuse given that 65% to 70% clarification of the extract was achieved with regenerated CaX zeolite.

Conventional extraction assisted by ultrasound and microwave was tested for the same purpose [36]. In this study three types of conventional extraction were described: cold (25 °C) for 12 h; extraction with ultrasound at (35 ± 5) °C for 30 min; and microwave-assisted extraction (MAE), performed at different wattages (from 20 W to 160 W) at time intervals of between 30 s and 5 min and at temperatures of between 10–90 °C. Optimum MAE conditions were found to be 80 W, for 1 min at 50 °C. In this study, the MAE yielded 8.64% and 2.34% of stevioside and rebaudioside A, respectively, while conventional and ultrasound techniques yielded 6.54% and 1.20%, and 4.20% and 1.98% of stevioside and rebaudioside A, respectively. The authors observed that the efficiency of extraction using PHWE (pressurized hot water extraction) or MAE was comparable or superior to that obtained by heat reflux extraction.

On the other hand, extraction by the supercritical fluid method has been selected and studied by several authors [15, 37]. In these studies, glycosides from *Stevia* leaves were obtained by supercritical fluid extraction, through a two-step process: firstly, extraction of CO_2 at 200 bar and 30 °C, and, secondly, extraction of CO_2 and water. The general extraction curves for the Stevia and CO_2 system showed a typical form and were successfully described [37]. Other studies mention the Japanese patent that provides information about the supercritical fluid extraction method used to obtain Stevia glycosides with carbon dioxide and co-solvents such as methanol, ethanol and acetone [38]. In other studies, the authors produced a statistical design to evaluate the effect of different pressures (150–350 bar), temperatures (40–80 °C) and the concentration (0–20%) of the ethanol-water mix (70:30) as the solvent, with a CO_2 flow of 15 g·min^{-1} for 60 min [15]. The optimum extraction conditions, according to the results, were 211 bar, 80 °C and 17.4% of solvent, which gave 36.66 mg·g^{-1} of stevioside and 17.79 mg·g^{-1} of rebaudioside A. The composition of the extract was similar to that obtained using conventional extraction methods with water (64.49 mg·g^{-1}) and a little higher than that obtained with ethanol (48.60 mg·g^{-1}). The results also demonstrated that stevioside yield is not significantly improved by increasing the pressure above 300 bar.

Enzymatic extraction was also used successfully [25]. In this investigation, the authors studied different extraction parameters, notably duration, temperature, the enzymes used and the concentration of each one. The enzymes used for extraction were: pectinase, cellulase and hemicellulose. They were tested at five concentrations (0.5%, 1%, 2%, 3% and 4%, w / v), under different temperature conditions as follows (35, 45 and 55) °C for pectinase; (40, 50 and 60) °C for cellulase; and (50, 60 and 70) °C for hemicelullase, and over different time periods [(15, 30 and 45) min]. The results demonstrated the feasibility of enzymatic extraction, which, according to the authors, is more efficient than conventional methods using solvents. The treatment which gave the highest yield of stevioside was 1 h at 60 °C, with hemicellulase.

Similarly, the effect of chitosan as a flocculating agent during the clarification of an aqueous extract of *Stevia* leaves was investigated [39]. Extraction was done at a leaf:water ratio of 1:10 (g:mL). The authors investigated the following conditions during the flocculation step: solution of acetic acid with 1% chitosan and dry *Stevia* leaves 2:1 (mL:g), pH 6, flocculation temperature 45 °C and flocculation time 4 h. A clarification rate of 86.63% was obtained with a loss of stevioside of only 6.89%. The authors concluded that flocculation of the extract with chitosan represents an efficient alternative, with yields comparable to those obtained by separation methods with membranes. In addition, no special equipment is needed and the waste materials are easily biodegraded, thus respecting environmental safety issues.

Lastly, based on a large number of patents [40–42, among others] which are deepened extraction and purification processes, we propose a general scheme for obtaining a steviol glycoside extract powder from leaves of *Stevia* [based on 13, 43] *(figure 2)*. In relation to the purification methods, amongst these, the membrane filtration technique is notable in that it manages to achieve adequate yields, without resorting to intense treatments, thus representing a 'green', environmentally friendly option. The most salient results obtained during applications of this particular technique were synthesized (*table II*).

6. Uses and applications in the food industry

From the above-mentioned considerations, it is clear that the addition of steviol glycosides can increase the palatability and enjoyment of food by improving flavors and smells [3]. Proof of this is the growing number of Stevia-based products on the world market, such as drinks, table-top sweeteners, candy and other processed foods, personal hygiene products and various Japanese delicacies.

Specifically, Stevia extracts have been used to sweeten low-calorie soft drinks, soy sauce, dried seafood, candy, ice cream, chewing gum and yogurt in several countries, but principally Japan, Korea and Brazil [28].

Information about the proportions of these sweeteners used in each type of product is available from the literature [48]. We listed some of the recommended values (*table III*).

There have been several investigations undertaken aimed at evaluating the potential of Stevia as a sweetener of specific products. For example, Lisak *et al.* prepared

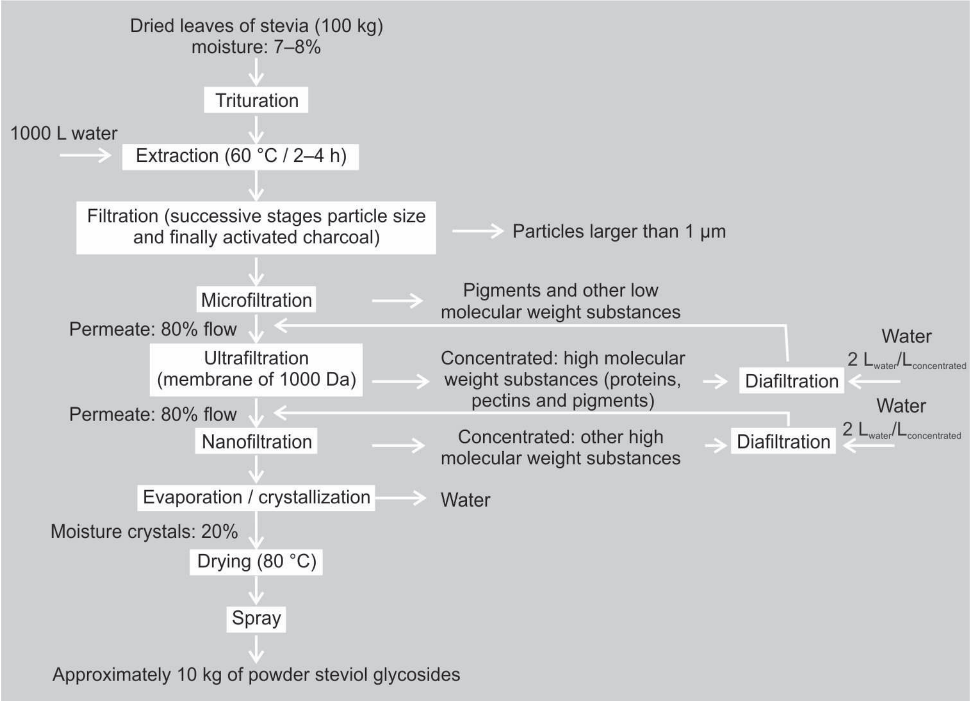

Figure 2.
General scheme for the preparation of a purified mixture of steviol glycosides from *Stevia* leaves by the membrane filtration technique [based on 13, 43].

strawberry-flavored yogurt sweetened with either sucrose, Stevia or equal proportions of sucrose and Stevia, this last a pure extract with 90% stevioside content, at three different concentrations [49]. According to the results given by the sensory panel judges, the authors determined that 6 g of Stevia is equivalent to 1000 g of sucrose. In taste tests, the yogurt sweetened with 4.5 g·100 g^{-1} of equal parts of sucrose and Stevia obtained the highest sensory acceptance scores. The apparent viscosity of the finished products was not affected by the addition of Stevia. Furthermore, after 7 days of cold storage, the degree of sweetness of all the different formulations tested remained the same as that of fresh samples (made the day before) of products prepared with sucrose.

Another study evaluating Stevia-sweetened yogurt was carried out by Guggisberg *et al.* [50]. Yogurt made with 8% sugar was replaced by Stevia, and combinations of Stevia with other sweeteners. Neither Stevia nor the other two commercial sweeteners used had any negative effects on the yogurt-making process or the pH, and did not significantly change the fermentation time or the generation of the casein network. However, the yogurt made with Stevia only had an unpleasant taste and thus could not be recommended [50].

Nevertheless, the yogurt made with a combination of Actilight (a commercial mixture of short-chain fructo-oligosaccharides) and Stevia showed a similar profile to the variant containing 8% sucrose. The authors concluded that low-calorie yogurts could be manufactured using commercial sweeteners including Stevia without modifying standard technological procedures.

A similar study, but this time applied to cake, assessed the rheological and microstructural properties and the final quality of cakes made by replacing sugar with stevioside (sweetener containing 80% stevioside) and liquid sorbitol [51]. The addition of hydrocolloids, emulsifiers and debittered fenugreek seed powder was also considered. The results showed that the addition of stevioside did not change the amylographic viscosity of wheat flour batter during heating and cooling, unlike sucrose, which increases this property. The authors concluded that it is possible to replace sugar with stevioside using this cake recipe while

Table II.
Studies related to the use of filtration membranes for the extraction of steviol glycosides.

Aim of the investigation	Study description	Results obtained	Reference
Clarification and purification with membranes	Three [(0.05, 0.1 and 0.2) mm] ceramic membranes were used at (2, 4 and 6) bar and extracts were obtained using the tangential filtration process	The 0.05-mm membrane at 2 bar gave yields of 97.1% stevioside and 94.7% rebaudioside	[14]
Effect of pH and temperature on a membrane separation process	The effect of temperature and pH on the separation and refining of glycosides, employing a multiple-stage membrane process (MF, UF and NF), was studied	pH did not affect the yield of extracted sweeteners; however, small quantities of components that contribute color were extracted at low pH	[43]
Ultrafiltration membrane	Membranes of (5, 10, 30 and 100) kDa were evaluated in order to select the most efficient size of ultrafiltration membrane	The 30-kDa membrane was the most efficient, giving the highest flow of the permeated liquid at a transmembrane pressure of 414 kPa and 1800 rpm	[5]
Purification with UF	Integrated MF, UF and NF processes with different commercial membranes and lab–made membranes were evaluated	High permeability was achieved but only at low polymer concentrations (27% PES). The methods used gave 30% yields of steviosides with 37% purity	[13]
Modeling of membrane ultrafiltration processes	A model using a gel layer for controlling the concentration of extract in an ultrafiltration cell was proposed	There was a favorable effect gained by lowering the concentration polarization of the cross–flow filtration	[44]
Modeling of membrane ultrafiltration processes	A mathematical model that enabled the estimation of the permeate flux, as well as the flow profiles and drop in concentration of steviosides during ultrafiltration, was proposed	The diffusivity of the gel-forming material of the Stevia extract was $(3.7 \pm 0.8) \times 10^{-11} m^2 \cdot s^{-1}$ and gel concentration was $(51.5 \pm 1.5)\ kg \cdot m^{-3}$	[45]
Cross-flow ultrafiltration	The effect of the operating conditions during cross-flow UF, using two methods (the total recycle mode and the batch concentration mode) was investigated	The general process proposed (UF + NF) achieved a yield of 60% of extract. Maximum recovery was obtained at 1241 kPa and 1500 rpm	[46]
Ultrafiltration	Different sized membranes with molecular weights of (5, 10, 30 and 100) kDa were studied	The 30-kDa membrane was the membrane best suited for clarification	[47]
Ultrafiltration and nanofiltration	A simple process for the purification and concentration of glycosides by a UF (30 kDa membrane at a feed pressure of 200–500 kPa) was proposed	The purification process gave a stevioside yield of $9.05\ g \cdot 100\ g^{-1}$ of Stevia leaves, and $0.2\ g \cdot 100\ g^{-1}$ of rebaudioside A, with a purity of total steviosides of 97.66%. This process also improved sweetness and the flavor profile.	[28]

Table III.
Concentrations of rebaudioside A in various products [1].

Product	Range ($mg \cdot kg^{-1}$ or $mg \cdot L^{-1}$)
Carbonated soft drinks	50–600
Still beverages	50–600
Powdered soft drinks	200–200
Chewing gum	300–6000
Dairy products	150–1000
Edible gels	200–1000
Nutraceuticals	200–1000
Pharmaceuticals	50–1000

maintaining the rheological properties of the final product.

Another study investigated the physical properties of cocoa powder drinks prepared with different fat contents and different sweeteners including Stevia extract [52]. The authors evaluated the bioactive content (content of polyphenols and antioxidant capacity) and the sensory properties of prepared cocoa drinks. The results showed that the type of sweetener used did not affect the polyphenolic constituents of the cocoa mixtures prepared. The results of the sensory analysis revealed a preference for cocoa drinks made with the sweeteners (aspartame/acesulfame K and Stevia extract) and a significant difference in the sensory attributes between the experimental mixtures and the control.

Finally, Mogran and Dashora determined the amount of Stevia extract (obtained by boiling *Stevia* leaf powder in water) and other commercial sweeteners required to produce a degree of sweetness equivalent to sugar [53]. Once the amounts required to achieve the desired sweetness had been determined, eleven products were elaborated: milk, coffee, tea, gajar halwa, milkshake, kheer, curd, lemon water, custard, halwa and lapsi, replacing the sugar in the recipes with either Stevia extract or one of the other commercial sweeteners. The results revealed that 1.5 mL of Stevia extract in 100 mL of liquid was equivalent to 5 g of sugar. The recipes prepared using Stevia were more acceptable than the other sweeteners tested, as indicated by the fact that they were given the highest sensory acceptance scores by members of the panel (7.67–7.90), thus occupying first place ahead of the artificial sweeteners tested. They were also statistically comparable in taste to sugar (sugar scores: 7.47–8.47) ($p > 0.001\%$) in the case of coffee, halwa, milk drinks, kheer and lapsi, and were scored as tastier for the rest of the products evaluated.

All of the above indicate the huge potential of Stevia as a realistic alternative to sugar in the products investigated. Not least because in addition to delivering similar physical and sensory properties, it also provides beneficial health effects for consumers.

7. Formulation of fruit juice sweetened with Stevia

A considerable number of studies have examined the use of sweeteners containing Stevia in the manufacture of fruit juices. One of these, carried out by Sharoba *et al.*, determined the rheological properties of two nectars, the first based on papaya and the second on apricot [4]. Both nectars were sweetened with aspartame and stevioside, to which various food hydrocolloids (guar gum, xanthan gum and arabic gum) were added. The liquids were characterized as being pseudoplastic with elastic limits at different temperatures [(5, 25, 50 and 75) °C]. Both sweeteners, however, lowered the pseudoplasticity, thus producing a decrease in the consistency index, plastic viscosity (or the resistance of fluid to flow) and shear stress.

As regards the sensory evaluation, all samples of both nectars, including the control, obtained medium/high scores. It should be noted that both the apricot and the papaya nectars which contained only the sweeteners (without the addition of any of the hydrocolloids) were given the lowest scores compared with the rest of the samples.

Another study assessed the sensory properties of peach nectar made with sucrose and other sweeteners (aspartame, cyclamate:saccharin mix 2:1, Stevia, sucralose and acesulfame-K) by means of a quantitative descriptive analysis [2]. Once the ideal concentration of each sweetener, that is, the concentration of the sweetener equivalent to sucrose sweetness, was determined, the sensory attributes of each sample were evaluated. The results show that the different formulations were not statistically different ($P \leq 0.05$) as regards yellow coloring, cloudy appearance and brightness. In spite of this, peach nectar sweetened with Stevia received the highest scores for the following negative attributes: grass aroma, bitterness, residual bitterness and residual sweetness; aspects which significantly influenced its acceptance in consumer tests. In terms of acidity, astringency, visual viscosity and body, however, no differences ($P \leq 0.05$) among samples were found. Thus, Stevia-sweetened nectar showed similar characteristics to nectars sweetened with sucrose.

A similar investigation sensorially evaluated industrialized pineapple juice, sweetened with sucralose, aspartame, Stevia (pure commercial extract), cyclamate/saccharin and sucrose [54]. The flavor profile obtained showed that juice made with Stevia had a sour and bitter aftertaste, demonstrating that Stevia may not be the appropriate sweetener for this particular juice formulation. In addition, residual sweetness, sweet flavor, spiciness and metallic taste were cited for these samples, but by very few tasters.

These same sweeteners were also studied in guava juice [55, 56]. As regards sweetness, guava juice sweetened with sucrose or aspartame achieved similar acceptance ratings (81%), while samples sweetened with Stevia (extract obtained from leaves) and cyclamate/saccharine were given higher scores for negative attributes, positioning them in the rejection zone as per the hedonic scale used: "I have a moderate dislike to these drinks" and "I don't like these drinks very much". The authors indicate that the matrix (guava nectar) did not affect the perception of sweetness of any of the sweeteners. Similar results were reported by Cavallini and Bolini, who compared the temporal perception of sweetness, bitterness and flavor in reconstituted mango juice sweetened with sucrose, cyclamate:saccharin 2:1, aspartame, sucralose and Stevia [57]. These authors concluded that, of all the sweeteners, aspartame showed a temporal profile closest to that of sucrose for the majority of the parameters tested. The juice made with Stevia, however, was the least similar to sucrose.

From these studies, it can be seen that many products sweetened with Stevia extract tend to have a bitter residual taste [55–57]. This may be attributed to the high proportions of stevioside glycoside contained in the mixture of leaf components obtained during the extraction process. This negative element is eliminated when plant varieties with higher concentrations of rebaudioside A are used, or simply by using steviol glycosides in pure form, due to their superior flavor profile [6]. Finally, it is important to highlight that sensory responses to formulated products are directly influenced by the food matrix used. Thus, each particular fruit juice sweetened with Stevia should be evaluated in depth in order to determine whether or not the aforementioned unpleasant residual taste can be perceived.

8. Conclusions and future outlook

Judging by the published literature, the use of steviol glycosides as sweeteners is an area that requires much further research, due to both their high appeal and commercial potential. This is very important, not only for manufacturers of industrial fruit juices, but also in general for various food applications. In this context, much research is still needed, not only to develop and optimize steviol glycoside extraction, but also to improve the taste of products sweetened with these compounds. Thus, the continued evaluation of these ingredients as regards aspects such as the intensity, persistence of sweet taste and absence of other residual flavors is necessary in order to meet the demands of today's consumers and ensure their acceptance, preference and choice by the general public.

Stevia-sweetened juices that have been evaluated so far (pineapple, peach, guava, apricot and papaya) presented the aforementioned unpleasant aftertaste. It is therefore necessary to investigate the most effective treatment to prevent its presence, especially in the case of citrus fruit juices that demand a greater proportion of sweetener. It should be tested with other fruits that naturally contain antioxidants that prevent the oxidation that causes the aforementioned aftertaste.

On the other hand, according to the authors, the addition of *Stevia* leaves in fruit juice extraction and clarification should also be considered in order to evaluate possible interactions between the food matrix (juice) and components responsible for the aftertaste.

Furthermore, and given that there is currently a sizeable and growing market for the commercialization of Stevia-containing products, the above-mentioned optimization of production and processing should be undertaken concurrently in order to avoid limitations in the supply of steviol glycosides, an aspect that could restrict their extensive use in the demanding future that lies ahead.

Finally, the patterns of use of Stevia and its approval by the major international regulatory organizations, who have confirmed both the safety of this product for human consumption and its stability over time, point towards its development as an ubiquitous sugar alternative. It is expected that steviol glycosides will be used mainly in the manufacture of beverages, along with other traditional foods (such as dairy products, bread and cakes, confectionery, etc.), table-top sweeteners, functional food and beverages, and nutritional supplements, in addition to their use in personal care products (such as toothpaste) and as an active pharmaceutical ingredient or excipient.

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Resumen de las principales propiedades de los esteviol glucósidos y su interés en la industria alimentaria.

Resumen – Introducción. En las últimas décadas, se ha incrementado el interés por desarrollar edulcorantes naturales, no calóricos, saludables y similares a la sacarosa. Una de las alternativas más prometedoras la constituye los esteviol glucósidos, compuestos de gran dulzor extraídos de las hojas de *Stevia rebaudiana* Bertoni. En esta revisión, se describen las particularidades de la mencionada planta. **Características y propiedades**. Se puntualizan los principales Steviol glucósidos reportados en la bibliografía, así como sus principales características y propiedades. **Toxicidad y legislación**. Se expone el panorama actual sobre la toxicidad y el marco legal de los mencionados endulzantes. **Técnicas de extracción**. Se describen las técnicas desarrolladas para su extracción y purificación; entre ellas, la extracción convencional con agua/solventes, intercambio iónico, extracción enzimática, por fluido supercrítico y con especial atención a la filtración por membranas; por sus ventajas comprobadas en la purificación del extracto acuoso obtenido por lixiviación de las hojas. **Aplicaciones**. Se presentan diversas evidencias relacionadas con la aplicación de esteviol glucósidos en la industria de alimentos, haciendo énfasis en su utilización para la formulación de jugos de frutas. **Conclusiones**. Se proyectan, según los juicios de los autores, las perspectivas futuras de estos edulcorantes. Se destaca la necesidad de investigación en el área, por su gran atractivo y potencial comercial, la cual debe estar enfocada en la evaluación continuada en lo que respecta a la intensidad, persistencia de sabor dulce y la ausencia de otros sabores residuales, con el fin de asegurar su aceptación, preferencia y elección por parte del público en general.

***Stevia rebaudiana* / hojas / extracción / glicosidos / edulcorantes / steviosido / industria alimentaria / jugo de frutas**