

## STEVIOLE GLYCOSIDES Chemical and Technical Assessment

Revised by Paul M. Kuznesof, Ph.D. for the 68<sup>th</sup> JECFA  
(Original prepared by Harriet Wallin for the 63<sup>rd</sup> JECFA)

### 1. Summary

The crushed leaves of the stevia plant, *Stevia rebaudiana* (Bertoni) are extracted with hot water and the additive is recovered from the aqueous extract using only alcohols and ion-exchange resins for the isolation and purification of the desired product. The commercial material contains at least 95% of total (seven) steviol glycosides (dried basis). Stevioside and rebaudioside A are the component glycosides of principle of interest because of their sweetening property. The newly revised specifications cover a range of compositions that include product of at least 95% stevioside or at least 95% rebaudioside A. Steviol glycoside preparations are white to light yellow powders that are freely soluble in water and ethanol. The powders can be odourless or have a slight characteristic odour. Water solutions are 200 to 300 times sweeter than sucrose. On the basis of results from thermal and hydrolytic stability studies on steviol glycosides and consideration of several summaries from the literature on the stability of stevioside and rebaudioside A, the 68<sup>th</sup> JECFA agreed that steviol glycosides is sufficiently thermally and hydrolytically stable for use in foods, including acidic beverages, under normal conditions of processing and storage. Preparations of steviol glycosides are reported to be used principally as sweeteners in fruit and milk-based drinks, desserts, yoghurts, confectionaries, delicacies, and pickles. The use of a stevia extract as a table-top sweetener is well known.

### 2. Introduction

Water extracts of the crushed leaves of the stevia plant, the perennial shrub *Stevia rebaudiana* (Bertoni) Bertoni of the family *Asteraceae* (*Compositae*), have been used for many years as a sweetener in South American and Asian countries. These substances currently are not permitted as conventional food ingredients or additives with a sweetener function in the United States nor have they yet been deemed acceptable for such use by the European Commission. However, the leaves of the stevia plant or extracts have been legally marketed in the United States as dietary ingredients in dietary supplements.

Stevia extracts generally contain a high percentage of the glycosides of the diterpene steviol. Stevioside and rebaudioside A are the principal sweetening compounds and are generally accompanied by smaller amounts of other steviol glycosides.

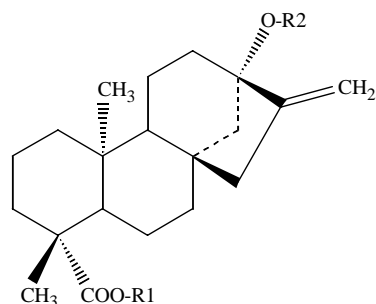
Based on the data and information received by the 63<sup>rd</sup> JECFA (2004), the Committee assigned a temporary ADI of 0-2 mg/kg bodyweight (bw) (expressed as steviol) for "steviol glycosides" and established tentative specifications. The temporary ADI and specifications related solely to material containing not less than 95% glycosylated derivatives of steviol, which, in addition to stevioside and rebaudioside A (summed content to be not less than 70% of total steviol glycosides), included rebaudioside C and dulcoside A, as well as minor amounts of rubusoside, steviolbioside, and rebaudiosides B, D, E, and F.

In order to remove the temporary designation from the ADI and the tentative designation from the specifications, the Committee required additional information on certain pharmacological effects in humans and analytical data on and a method of analysis for those steviol glycosides *not* identified in the specifications. Further, the Committee requested information on the composition of the non-steviol glycoside fraction, on quantities of residual solvents from the manufacturing process, on hydrolytic stability of the additive in acidic foods and beverages.

At the 68<sup>th</sup> JECFA, the Committee reviewed responses addressing the outstanding issues from the 63<sup>rd</sup> JECFA. Although the Committee maintained the temporary ADI of 0-2 mg/kg bw (expressed as steviol), the tentative designation for the specifications were removed and full specifications were prepared.

### ***3. Structures of steviol glycosides***

The structures and Chemical Abstracts Service registry numbers for steviol and its glycosides that are the main sweetening agents of the additive steviol glycosides are given in Figure 1.



	Compound name	C.A.S. No.	R1	R2
1	Steviol	471-80-7	H	H
2	Steviolbioside	41093-60-1	H	$\beta$ -Glc- $\beta$ -Glc(2→1)
3	Stevioside	57817-89-7	$\beta$ -Glc	$\beta$ -Glc- $\beta$ -Glc(2→1)
4	Rebaudioside A	58543-16-1	$\beta$ -Glc	$\beta$ -Glc- $\beta$ -Glc(2→1)   $\beta$ -Glc(3→1)
5	Rebaudioside B	58543-17-2	H	$\beta$ -Glc- $\beta$ -Glc(2→1)   $\beta$ -Glc(3→1)
6	Rebaudioside C (dulcoside B)	63550-99-2	$\beta$ -Glc	$\beta$ -Glc- $\alpha$ -Rha(2→1)   $\beta$ -Glc(3→1)
7	Rebaudioside D	63279-13-0	$\beta$ -Glc- $\beta$ -Glc(2→1)	$\beta$ -Glc- $\beta$ -Glc(2→1)   $\beta$ -Glc(3→1)
8	Rebaudioside E	63279-14-1	$\beta$ -Glc- $\beta$ -Glc(2→1)	$\beta$ -Glc- $\beta$ -Glc(2→1)
9	Rebaudioside F	438045-89-7	$\beta$ -Glc	$\beta$ -Glc- $\beta$ -Xyl(2→1)   $\beta$ -Glc(3→1)
10	Rubusoside	63849-39-4	$\beta$ -Glc	$\beta$ -Glc
11	dulcoside A	64432-06-0	$\beta$ -Glc	$\beta$ -Glc- $\alpha$ -Rha(2→1)

Figure 1. Structures of steviol and related glycosides. Glc, Xyl, and Rha represent, respectively, glucose, xylose, and rhamnose sugar moieties.

#### 4. Description

Steviol glycoside preparations are white to light yellow powders that are freely soluble in water and ethanol. The powders can be odourless or have a slight characteristic odour. Water solutions are 200 to 300 times sweeter than sucrose.

#### 5. Manufacture of steviol glycosides

The basic raw materials for manufacturing steviol glycoside preparations are the crushed leaves of the perennial shrub *Stevia rebaudiana* (Bertoni) Bertoni of the family *steraceae* (*Compositae*).

Conventional extraction processes to obtain steviol glycoside-containing extracts that are described in the literature follow similar methodologies: Stevia leaves are extracted with hot water or alcohols. In some cases, the leaves are pretreated with non-polar solvents, such as chloroform or hexane to remove essential oils, lipids, chlorophyll, and other non-polar substances. The extract is clarified by precipitation with salt or alkaline solutions, concentrated, and re-dissolved in methanol for crystallization of the glycosides. Additionally, reports of the use of supercritical fluid extraction (SCFE) using carbon dioxide to ultimately obtain product have appeared in the literature.

The specified additive, however, following hot-water extraction of crushed stevia leaves, is obtained from the aqueous extract using only alcohols and ion-exchange resins for the isolation and purification of the desired product. The commercial product may be recovered by spray-drying.

The Committee, at its 68<sup>th</sup> meeting reviewed information on additional purification steps included in the manufacturing process of steviol glycosides considered by the 63<sup>rd</sup> JECFA. These steps included further recrystallization and separation steps; but, no new solvents were introduced. The purity of the commercial material is not less than 95% of total (seven) steviol glycosides (on the dried basis); these include substances 2-6, 10, and 11 in Figure 1, above.

## **6. Chemical characterization**

### *6.1 Composition of the food additive*

The composition of stevia extracts depends on the composition of the leaves, influenced by soil and climate, and on the extraction and purification processes used.

In response to the call for data on “stevioside” for the 63<sup>rd</sup> meeting of the Committee, submissions from several countries showed that the main components of the commercially available extracts of stevia are stevioside and rebaudioside A, in various amounts ranging from about 10-70% stevioside and 20-70% rebaudioside A. The information indicated that most commercial products contained more than 90% steviol glycosides with the two main steviol glycosides comprising about 80% of the material. The 63<sup>rd</sup> JECFA required that the summed content of stevioside and rebaudioside A was not less than 70% and established a minimum purity of 95% total steviol glycosides. Analytical data showed that most of the remaining 5% could be accounted for by saccharides other than those associated with the individual steviol glycosides.

Noting that the additive could be produced with high purity (at least 95%) and that all the steviol glycosides hydrolyze upon ingestion to steviol, on which the temporary ADI is based, the 68<sup>th</sup> JECFA decided it was unnecessary to maintain a limit for the sum of stevioside and rebaudioside content. The Committee recognized that the newly revised specifications would cover a range of compositions that could include, on the dried basis, product that was at least 95% stevioside or at least 95% rebaudioside A.

### *5.2 Possible impurities*

Impurities occurring in extracts of stevia leaves are typical plant materials, such as pigments and saccharides. One literature study reports identification of the following substances in the non-glycosidic fractions of extracts of stevia leaves, obtained using SCFE: spathulenol; decanoic acid;

8,11,14-ecosatrienoic acid; 2-methyloctadecane; pentacosane; octacosane; stigmasterol; b-sitosterol; a- and b-amyrine; lupeol; b-amyrin acetate; and pentacyclic triterpene. These highly non-polar substances represented 56% of the total non-glycosidic extracts, 44% remaining unidentified.

The specified additive (>95% total steviol glycosides) will contain, in addition to saccharides other than those associated with the individual steviol glycosides, residual extraction/recrystallization solvent and possibly residues of ion-exchange resins used in the manufacturing process. The level of the non-glycosidic fraction, because of its highly non-polar character, can be considered insignificant in the additive.

The limit of not more than 4% loss-on-drying established by the 63<sup>rd</sup> JECFA was increased to not more than 6% by the 68<sup>th</sup> JECFA and the maximum limit for residual solvents of 100 mg/kg also established by the 63<sup>rd</sup> JECFA was raised to 200 mg/kg.

Maximum limits of 1 mg/kg for both arsenic and lead were maintained.

### *6.3 Analytical methods*

Different methods, mainly liquid chromatographic methods, are currently available for the identification and determination of the principal steviol glycosides.

## ***7 Functional uses and reactions/fate in foods***

Preparations of steviol glycosides have been reported to be used principally as sweeteners in fruit and milk-based drinks, desserts, yoghurts, confectionaries, delicacies, and pickles. The use of a stevia extract as a table-top sweetener is well known; in the United States it is legally marketed only as a “dietary supplement,” but, consumers are known to purchase the product for its sweetening properties.

Stevioside and rebaudioside A are reasonably thermally stable under the elevated temperatures used in food processing and do not undergo browning or caramelization when heated. In response to a request by the 63<sup>rd</sup> JECFA for information on the hydrolytic stability of steviol glycosides in acidic foods, the Committee received results of thermal and hydrolytic stability studies for the specified material: It appears that citric acid solutions (pH 2-4) of steviol glycosides (ca. 1000 mg/l; ca. 29% stevioside, 62% rebaudioside A) are highly stable for at least 180 days at 20°. At elevated temperatures (80°, in water, 8 h), however, the same samples showed 4% and 8% decomposition at, respectively, pH 4.0 and 3.0. At 100°, decomposition was expectedly higher: 10% and 40% at, respectively, pH 4.0 and pH 3.0. Also, at 100° decomposition was 4% at pH 6.0, but increased to about 16% at pH 8.0. And in an acidic beverage (pH 3.8) held at 24° for one year, essentially no decomposition of steviol glycosides (ca. 94% rebaudioside) was noted. Isosteviol (Figure 2) was identified as a decomposition product in the tested samples:

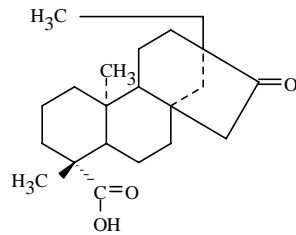


Figure 2. Isosteviol (C.A.S. no. 27975-19-5)

The Committee also had available a summary of literature studies that addressed the stabilities of stevioside and rebaudioside A, which it considered relevant to its evaluation because the specified material includes products that may be 95% stevioside or 95% rebaudioside. Unfortunately, information on the purities of the substances used in these studies was not provided. The studies are summarized as follows:

- 1) Refluxing stevioside (5 h; 0.4% hydrochloric acid (pH 1.16, aq. methanol)): identifiable hydrolysis products: 49% steviol, 29% steviolbioside, 4% 13-O- $\beta$ -D-glucopyranosyl-steviol, and 19% 13-O- $\beta$ -D-glucopyranosyl-steviol- $\beta$ -D-glucopyranosyl ester.
- 2) Heating stevioside or rebaudioside A (citric or phosphoric acid solutions; 5.7 days): no decomposition at 60°; at 100°, decomposition products noted, but not identified. No steviol was observed.
- 3) Stevioside (aqueous acid, pH 2.5 and 3.0; 80° and 100°): After one hour, maximum decomposition (10%) noted at pH 2.5 and 100°. No information on the acid used.
- 4) Stevioside and rebaudioside A (650 mg/l; 100°): Decomposition in neutral solutions after 13 hours; 40% decomposition in acid solutions (citric acid, pH 2.6; phosphoric acid, pH 2.4) after 4 hours.
- 5) Stevioside (130 mg/l; citric acid; room temperature; 6 months): 2.5% loss at pH 4.0; 10% loss at pH 3.0.
- 6) Stevioside (1000 mg/l; pH 2.6 - citric acid solution; 4° and 22°): stable for up to 4 months. Rebaudioside A (22°; pH 2.6): slightly less stable than stevioside; some decomposition after three months. At 37°, both substances begin to decompose after 2 months; ca. 15% decomposed at 4 months. These data also suggest that, in citric acid solutions, stevioside is more thermally stable than Rebaudioside A.
- 7) Rebaudioside A (1000 mg/L; phosphoric acid - pH 2.6) showed slightly greater thermal stability than stevioside (1000 mg/L; phosphoric acid - pH 2.6).
- 8) Stevioside (500 mg/l; room temp.; solutions of 10 g/l citric and phosphoric acids (pH: 2.1 and 1.6, respectively): decomposition begins after one month; stable in 5% acetic acid (pH 2.6). In the phosphoric acid solution (4 months), decomposition reached 75%; in citric acid, decomposition was 20%. The greatest stability was observed for the acetic acid solution.

The 68<sup>th</sup> JECFA concluded that steviol glycosides is sufficiently thermally and hydrolytically stable for food use, including acidic beverages, under normal conditions of processing/storage.

## 8 Use levels in foods

The steviol glycosides are currently used as a sweetener in a number of foods consumed in Asia and South America. Table 1 summarizes the information submitted to the JECFA at its 63<sup>rd</sup> meeting.

**Table 1. Food Use Levels Reported to the Committee  
Reported maximum use level (mg/kg)**

Beverages (soft drinks, fruit drinks)	500	Pickles	1000
Desserts	500	Delicacies	1000
Yogurt	500	Sweet Corn	200
Cold Confectionery	500	Breads	160
Sauces	1000	Biscuits	300

Additional uses include use as a table-top sweetener and as a sweetener for ready-to-eat-cereals.

## 9. References

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Chen, Junshi, Personal communication to Harriet Wallin, 5 January 2004.

*Extraction of Stevia Glycosides with CO<sub>2</sub> + Water, CO<sub>2</sub> + Ethanol, and CO<sub>2</sub> + Water + Ethanol.* [http://www.gireaud.net/stevia\\_extract\\_indus.htm](http://www.gireaud.net/stevia_extract_indus.htm) (Source cited as: Brazilian Journal of Chemical Engineering) accessed 14 April 2007.

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Riveros, T. V. J., 2003. Scientific-Technological Report on Stevioside for JECFA Evaluation. National Institute of Technology and Standardization (INTN), Asunción, Paraguay.

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The following three references were cited in *Extraction of Stevia Glycosides with CO<sub>2</sub> + Water, CO<sub>2</sub> + Ethanol, and CO<sub>2</sub> + Water + Ethanol* and relate to the composition of stevia extracts:

Pasquel, A., 1999. *Extração dos Glicosídeos da Estévia com Fluidos Pressurizados*, Ph.D. diss., Universidade Estadual de Campinas, Campinas, SP, Brazil.

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Tan, S., Shibuta, Y., and Tanaka, O., 1988. Isolation of sweetener from *Stevia rebaudiana*. Jpn. Kokai, 63, 177, 764.

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Chang, S. S., and Cook, J. M., 1983. Stability studies of stevioside and rebaudioside A in carbonated beverages. *Journal of Agricultural and Food Chemistry* 31, 409-412.

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