STEVIOL GLYCOSIDES

Chemical and Technical Assessment
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1 Introduction

Stevioside was evaluated for safety by the 51st meeting of the JECFA in 1998 (WHO TRS no. 891), but the Committee was unable to establish an ADI for the substance due to insufficient data. Additional data had been received for the evaluation of Stevioside by the 63rd JECFA in 2004.

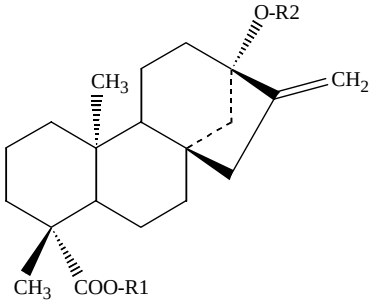
Water extracts of the crushed leaves of the stevia plant, the perennial shrub *Stevia rebaudiana* (Bertoni) Bertoni of the family *Asteraceae (Compositae)*, have been used for many years as a sweetener in some countries in South America and Asia. In the United States, however, although the leaves of the Stevia plant or extracts have been marketed as dietary ingredients in dietary supplements, these substances currently are not permitted as conventional food ingredients or additives with a sweetener function. In 2000, the European Commission did not accept *Stevia* or stevioside as a novel food based on opinions expressed on 17.6.1999 by the Scientific Committee on Food according to which the substance was not acceptable as a sweetener on the then presently available data.

Stevia extracts generally contain a high percentage of the glycoside diterpenes stevioside (CAS no. 57817-89-7) and rebaudioside A (CAS no. 58543-16-1), the principal sweetening compounds, and smaller amounts of other steviol glycosides. The composition of the extracts depends on the composition of the leaves, influenced by soil and climate, and on the extraction and purification processes used. The impurities occurring in extracts of the Stevia leaves are typical plant materials, such as pigments and saccharides.

Submission from countries in different parts of the world suggest that the main components of the commercially available extracts of stevia contain as the main components stevioside and rebaudioside A in various amounts ranging from about 10-70% stevioside and 20-70% rebaudioside A. It appears that most commercial products have a total steviol glycoside content of more than 90% with the the two main steviol glycosides making up about 80% of the material.

2 Nomenclature

As described above, extracts of *Stevia* have variable compositions. Structures of stevioside and related compounds are given in Figure 1. Steviol is the aglycone of all of the principle and secondary sweetener components (the glycoside terpenes).



	Compound name	R1	R2
1	Steviol	H	H

	Compound name	R1	R2
2	Steviolbioside	H	β-Glc-β-Glc(2→1)
3	Stevioside	β-Glc	β-Glc-β-Glc(2→1)
4	rebaudioside A	β-Glc	β-Glc-β-Glc(2→1) | β-Glc(3→1)
5	rebaudioside B	H	β-Glc-β-Glc(2→1) | β-Glc(3→1)
6	rebaudioside C (dulcoside B)	β-Glc	β-Glc-α-Rha(2→1) | β-Glc(3→1)
7	rebaudioside D	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1) | β-Glc(3→1)
8	rebaudioside E	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1)
9	rebaudioside F	β-Glc	β-Glc-β-Xyl(2→1) | β-Glc(3→1)
10	dulcoside A	β-Glc	β-Glc-α-Rha(2→1)

Figure 1. Structures of stevioside and related compounds. In rebaudioside D and E R1 is composed of 2 β-Glc-β-Glc(2→1). In rebaudioside A, B, C, D, E and F in group R2 an additional sugar moiety is added on carbon 3 of the first β-Glc. In rebaudioside F one β-Glc is substituted for by -β-Xyl. Glc and Rha represent, respectively, glucose and rhamnose sugar moieties.

The commercially available products under various names (Stevioside, Stevia extracts, Purified Stevia Extract etc.) vary in composition from one manufacture of sweetener product to another with respect to the relative ratios of the steviol glycosides and other constituents.

Extracts of stevia leaves manufactured with high levels of Rebaudioside A compared to levels of stevioside have been produced in Japan during the last twenty years. Four such products, namely Stevia extract, Stevia essence, Stevia powder, and Stevia sweetener, were included in "List of Food Additives Excluding Chemical Synthetics," published in 1989 by the Ministry of Health and Welfare, Japan.

There is no single common or trivial name established through common usage that is available to identify the specific commercial materials being evaluated at the current JECFA. In 1988 the 33rd JECFA developed guidelines (WHO TRS no. 776) for designating titles for specification monographs. Based on these guidelines the Committee named the specification monograph "Steviol glycosides" to indicate that the commercial products are mixtures of the steviol glycosides.

3 Description

Steviol glycosides preparations are white or slightly yellowish white crystalline odourless or having a slight characteristic odour, water soluble powders, which are 200 to 300 times sweeter than sucrose.

4 Manufacturing

4.1 Raw materials

The raw materials used in the manufacturing process of Steviol glycosides preparations are the crushed leaves of the perennial shrub *Stevia rebaudiana* (Bertoni) Bertoni of the family *Asteraceae (Compositae).* Various *a*lcohols and ion exchange resins are used in the manufacturing.

4.2 Methods of manufacture

Manufacturer from Paraguay

Material from South America may contain greater than 95% total carbohydrate, with stevioside content around 70% and rebaudioside A approximately 20%; minor steviol glycosides are likely not more than 5% total.

The basic stages of the extraction process of sweetener principles of *Stevia rebaudiana* leaves are:

Preparation of the aqueous extract

Preparation of the organic extract

Obtaining steviol glycosides

Preparation of the aqueous extract

The ground leaves of *Stevia rebaudiana* are mixed with hot water for 20-30 min. Subsequently, the aqueous extract is removed by draining, using pressure in order to achieve the maximum amount of extract. Several types of infusion/draining processes may be used. The extract is allowed to cool to room temperature. In order to remove impurity particles, the extract may be allowed to rest while particulate matter settles out or it may be centrifuged. The extract contains the sweetener principles, the plant pigments and other water-soluble components.

Preparation of the organic extract

The sweetener principles and the pigments are extracted with a mixture of butanol or isobutanol and a less polar solvent, such as benzene, chloroform or hexane.

Obtaining steviol glycosides

The organic phase is separated and concentrated until a solid mass is formed. The mass is dissolved in hot methanol. Steviol glycoside crystals form on cooling. The crystals are separated and washed with cold methanol, and finally recrystallized from methanol/water. The resulting material has a high degree of purity (97-98% steviol glycosides) and contains about 4% water.

Manufacturer from Japan

The manufacturing process used in Japan is reported to yield a product containing 80-88% of the four sweetening agents (stevioside, rebaudioside A, rebaudioside C and dulcoside A). Other steviol glycosides and saccharides are present at 5-10%.

The manufacturing process is presented as a flow sheet:

Process	Operating conditions
1. Dry *Stevia* leaves	Harvest; Leaves and stem dissociation
2. Extraction	Extract 2-3 times with water at 50-60°
3. Flocculation	Add calcium hydroxide and aluminium sulphate
4. Filtration	Filter press

5. Adsorption on resin	The steviol glycosides are adsorbed and then released with alcohol
6. Distillation	Alcohol vaporisation
7. Ion exchange	Cation and anion resin mix bed
8. Concentration	Vaporize water under reduced pressure
9. Micro filter	1 µm
10. Sterilization	120°, 1 min
11. Spray drying	Drying

5 Chemical characterization

5.1 Composition of the food additive

The chemical composition of steviol glycosides preparations varies considerably depending on the manufacturing process and also on the leaves used as raw material, as their composition varies with the temperature during the year of cultivation, the length of daylight during the growth period, and the time of harvest. Based on information, products on the market contain not less than 80% of the two principal and two secondary steviol glycosides, and the sum of the percentages of stevioside and rebaudioside A is not less than 70%. Other steviol glycosides are also present in minor amounts (5-10%). However, the composition (qualitative and quantitative) of the remaining fractions of the extracts has not been sufficiently characterized.

5.2 Possible impurities

The impurities occurring in steviol glycosides are due primarily to compounds extracted from the Stevia leaves. Analytical results on preparations support a setting of maximum limits of 1 mg/kg for both arsenic and lead.

5.3 Analytical methods

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

6 Functional uses and reactions/fate in foods

Steviol glycoside extracts are mainly used as a sweetener in the manufacture of fruit and milk drinks, desserts, yoghurt, cold confectionary, delicacies and pickles. Stevioside and Rebaudioside A are reasonably stable under the elevated temperatures used in food processing and do not undergo browning or caramelization when heated. No information on the hydrolytic stability of steviol glycosides in acidic foods was available to the Committee.

7 Use levels in foods

The steviol glycosides are used to sweeten a number of foods in Asia and South America. Table 1 summarizes the information submitted to the Committee.

Table 1. Food Use Levels Reported to the Committee

Food type	**Reported maximum use level (mg/kg)**
Beverages	500
Desserts	500

Yogurt	500
Cold Confectionery	500
Sauces	1000
Pickles	1000
Delicacies	1000
Sweet Corn	200
Bread	160
Biscuits	300

It is also known that stevia leaves are used to prepare a sweetened tea in a number of countries throughout the world. The levels of stevia glycosides in these teas would likely be lower than those found in Table 1.

8 References

JECFA Application, submitted by Japan

"List of Food Additives Excluding Chemical Synthetics," published in 1989 by the Ministry of Health and Welfare, Japan.

Jiménez Riveros, T. V., Scientific-Technological Report on Stevioside for JECFA Evaluation INTN National Institute of Technology and Standardization, Asinción, Paraguay, 2003

SCF (1999) Opinion on Stevioside as a Sweetener, adopted 17/6/1999.

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European Commission Decision 2000/196/EU, OJ L 61, p. 14, 8.3.2000.

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