Food and Agriculture Organization of the United Nations

**84th JECFA - Chemical and Technical Assessment (CTA), 2017**


# STEVIOL GLYCOSIDES

## Chemical and Technical Assessment (CTA)

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## *1. Summary*

Steviol glycosides are a group of compounds naturally occurring in the plant *Stevia rebaudiana* Bertoni sharing a similar molecular structure where different sugar moieties are attached to the aglycone steviol (an *ent*-kaurene-type diterpene). They include any compound containing a steviol backbone conjugated to any number or combination of the principal sugar moieties in any of the orientations naturally occurring in the leaves of *Stevia rebaudiana* Bertoni including, but not limited to, glucose, rhamnose, xylose, fructose, deoxyglucose, arabinose and galactose. The Committee has evaluated steviol glycosides at six previous meetings (51st, 63rd, 68th, 69th and 73rd and 82nd). Tentative specifications were initially established for steviol glycosides at the 63rd meeting pending the review of additional information related to the stability, manufacturing method and specifications of the products in commerce. Upon resolving the outstanding technical issues, specifications for steviol glycosides were finalized at the 68th meeting, with additional revisions made at the 69th and 73rd meetings as a result of further technical developments. The Committee at its 82nd meeting (JECFA, 2016), reviewed the information on steviol glycosides and noted that several new glycosides from the leaves of *Stevia rebaudiana* Bertoni have been isolated and identified. The Committee revised the specifications for steviol glycosides, made the specifications tentative and requested information on the following:

- Method of assay to replace the existing method and including as many steviol glycosides as possible (at least those listed in Appendix 1 of the specifications) in steviol glycoside mixtures, along with supporting validation information and chromatograms;
- Analysis results from a minimum of five batches for commercial samples, including supporting chromatograms

New specifications for Rebaudioside A, derived from Multiple Gene donors expressed in *Yarrowia lipolytica*, were also prepared at the 82nd meeting ((JECFA, 2016).

The Committee, at the 84th JECFA, reviewed the information on the assay methods as well as additional information on the isolation and identification of new steviol glycosides, resulting in different sugar orientations, and including two additional sugars, namely arabinose and galactose. The specifications were revised by replacing the assay method with a suitable method to quantify the major and most of the minor steviol glycosides. Information on the new steviol glycosides was included and the tentative status was removed.

**Steviol Glycosides from *Stevia rebaudiana* Bertoni** are produced from the crushed leaves of the stevia plant, *Stevia rebaudiana* Bertoni, by extraction with hot water and recovery from the aqueous extract using only alcohols and ion-exchange resins for the isolation and purification of the desired product. The products in commerce contain at least 95% of total steviol glycosides (on the dried basis) with a variable composition depending upon the composition within the leaves of the *Stevia rebaudiana* plant, which is influenced by both soil and climate, as well as manufacturing techniques (adsorption column) and conditions (temperature, pH).

**Rebaudioside A from Multiple Gene Donors Expressed in *Yarrowia lipolytica*** is produced by fermentation of a genetically modified strain of *Yarrowia lipolytica* to express the *Stevia rebaudiana* metabolic pathway. It is at least 95% rebaudioside A (anhydrous basis).

Commercial products containing steviol glycosides are white to light yellow powders that are freely soluble in mixtures of water and ethanol (50:50). 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$^{th}$ 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 steviol glycosides as a table-top sweetener is also well known.

## *2. Structures of steviol glycosides*

Steviol glycosides can be categorised into separate groups based on the type of glycosidic residues linked to the steviol backbone: glucosyl steviol (only glucose residues); rhamnosyl steviol (rhamnose and glucose residues); xylosyl steviol (xylose and glucose residues); fructosyl steviol (fructose and glucose residues); deoxyglucose steviol (deoxyglucose and glucose residues); arabinosyl steviol (arabinose and glucose residues); and galactosyl steviol (galactose and glucose residues).

The chemical information on steviol glycosides is given in Table 1. The Committee at its 73$^{rd}$ meeting (JECFA 2010), identified only glucose-, rhamnose-, and xylose-conjugated steviol glycosides. At the 82$^{nd}$ meeting (JECFA, 2016), fructose and deoxyglucose were also recognised as additional sugar conjugates of steviol. Since then, several new steviol glycosides were isolated and identified (Purkayastha, 2016): Five steviol glycoside compounds (*i.e*., rebaudioside W, rebaudioside W2, rebaudioside W3, rebaudioside Y and rebaudioside T) have been identified which are conjugated with the sugars, arabinose and galactose. Two diterpene glycosides (Rebaudiosides T and U), minor C-19 xylopyranosyl and arabinopyranosyl steviol glycoside were identified in the leaves of Stevia (Perera, 2017). It is possible that additional sugar moiety conjugates of steviol will be identified in the *Stevia rebaudiana* Bertoni plant in the future. The definition of the additive may be updated as and when new sugar moiety conjugates are identified and they may be included in its assay.

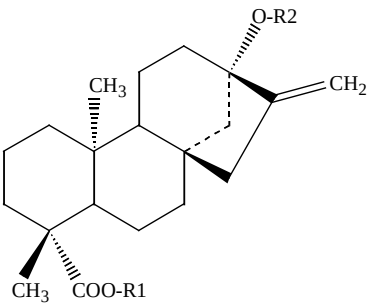


Figure 1. Structures of steviol and related glycosides. Steviol (R1 = R2 = H) is the aglycone of the steviol glycosides. Glc, Rha, Fru, deoxyGlc, Xyl, Ara and Gal represent, respectively, glucose, rhamnose, fructose, deoxyglucose, xylose, arabinose and galactose sugar moieties.

**Table 1: Steviol Glycosides Identified in *Stevia rebaudiana* Bertoni**

| Common Name | Trivial Name | $R_1$ | $R_2$ | Chemical Name | CAS Number | Chemical Formula | Formula Weight |
|---|---|---|---|---|---|---|---|
| **Group 1: Steviol + Glucose (SvGn)** | | | | | | | |
| Steviolmonoside | SvG1 | H | Glcβ1- | 13-[(β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid | 60129-60-4 | $C_{26}H_{40}O_8$ | 481 |
| Steviolmonoside A | SvG1 | Glcβ1- | H | 13-[(hydroxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester | 64977-89-5 | $C_{26}H_{40}O_8$ | 481 |
| Rubusoside | SvG2 | Glcβ1- | Glcβ1- | 13-[(β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester | 64849-39-4 | $C_{32}H_{50}O_{13}$ | 643 |
| Steviolbioside | SvG2 | H | Glcβ(1-2)Glcβ1- | 13-[(2-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid | 41093-60-1 | $C_{32}H_{50}O_{13}$ | 643 |
| Stevioside | SvG3 | Glcβ1- | Glcβ(1-2)Glcβ1- | 13-[(2-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester | 57817-89-7 | $C_{38}H_{60}O_{18}$ | 805 |
| Stevioside A Or Rebaudioside KA | SvG3 | Glcβ(1-2)Glcβ1- | Glcβ1- | 13-[(2-O-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid 4')-*O*-β-D-glucopyranosyl-deoxy-(1,2)-*O*-[ β-(-D-glucopyranosyl ester | 127345-20-4 | $C_{38}H_{60}O_{18}$ | 805 |
| Stevioside B | SvG3 | Glcβ(1-3)Glcβ1- | Glcβ1- | 13-[(2-O-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, *O*-β-D-glucopyranosyl-deoxy-(1,3)-*O*-[ β-D-glucopyranosyl ester | - | $C_{38}H_{60}O_{18}$ | 805 |
| Rebaudioside *B* | SvG3 | H | Glcβ(1-2)[Glcβ(1-3)]Glcβ1- | 13-[(2-*O*-β-D-glucopyranosyl-3-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid | 58543-17-2 | $C_{38}H_{60}O_{18}$ | 805 |

| Common Name | Trivial Name | $R_1$ | $R_2$ | Chemical Name | CAS Number | Chemical Formula | Formula Weight |
|---|---|---|---|---|---|---|---|
| Rebaudioside G | SvG3 | Glcβ1- | Glcβ(1-3)Glcβ1 | 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid(4')-*O*-β-D-glucopyranosyl ester | 127345-21-5 | $C_{38}H_{60}O_{18}$ | 805 |
| Rebaudioside *E* | SvG4 | Glcβ(1-2)Glcβ1- | Glcβ(1-2)Glcβ1- | 13-[(*O*-β- D-Glucoopyranosyl-(1,2)-*O*-[ β- D-glucopyranosyl)-oxy]-kaur-16-en-18-oic acid (4')-*O*-β-D-glucopyranosyl-deoxy-(1,2)-*O*-[ β-D-glucopyranosyl ester | 63279-14-1 | $C_{44}H_{70}O_{23}$ | 967 |
| Rebaudioside *A* | SvG4 | Glcβ1- | Glcβ(1-2)[Glcβ(1-3)]Glcβ1- | 13-[(2-*O*-β-D-glucopyranosyl-3-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester | 58543-16-1 | $C_{44}H_{70}O_{23}$ | 967 |
| Rebaudioside A2 | SvG4 | Glcβ1- | Glcβ(1-6)[Glcβ(1-2)]Glcβ1- | 13-[(6-O-β-D-glucopyranosyl-2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, **2-O**-β-D-glucopyranosyl ester | 1326217-29-1 | $C_{44}H_{70}O_{23}$ | 967 |
| Rebaudioside *D* | SvG5 | Glcβ(1-2)Glcβ1- | Glcβ(1-2)[Glcβ(1-3)]Glcβ1- | 13-[(2-*O*-β-D-glucopyranosyl-3-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl ester | 63279-13-0 | $C_{50}H_{80}O_{28}$ | 1129 |
| Rebaudioside L | SvG5 | Glcβ1- | Glcβ(1-6) Glcβ(1-2) [Glcβ(1-3)]Glcβ1- | 13-[(6-O-β-D-glucopyranosyl-2-O-β-D-glucopyranosyl-3-β-D-glucopyranosyl- β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl ester | 1220616-38-5 | $C_{50}H_{80}O_{28}$ | 1129 |
| Rebaudioside I | SvG5 | Glcβ(1-3) Glcβ1- | Glcβ(1-2) [Glcβ(1-3)]Glcβ1- | 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 3-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester | - | $C_{50}H_{80}O_{28}$ | 1129 |

| Common Name | Trivial Name | $R_1$ | $R_2$ | Chemical Name | CAS Number | Chemical Formula | Formula Weight |
|---|---|---|---|---|---|---|---|
| Rebaudioside I2 | SvG5 | Glcβ1- | Glcα(1-3) Glcβ(1-2) [Glcβ(1-3)]Glcβ1- | 13-[(3-O-β-D-glucopyranosyl-2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl- β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl ester | - | $C_{50}H_{80}O_{28}$ | 1129 |
| Rebaudioside I3 | SvG5 | [Glcβ(1-2) Glcβ(1-6)]Glcβ1- | Glcβ(1-2)Glcβ1- | 13-[(2-O-β-D-glucopyranosyl-O-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl-6-O- β-D-glucopyranosyl-β-D-glucopyranosyl ester | - | $C_{50}H_{80}O_{28}$ | 1129 |
| Rebaudioside Q | SvG5 | Glcβ1- | Glcα(1-4)Glcβ(1-2)[Glcβ(1-3)]Glcβ1- | 13-[(4-O-β-D-glucopyranosyl-2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl- β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl ester | - | $C_{50}H_{80}O_{28}$ | 1129 |
| Rebaudioside Q2 | SvG5 | [Glcα(1-2) Glcα(1-4)] Glcβ1- | Glcβ(1-2)Glcβ1- | 13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl-4-O-β-D-glucopyranosyl- β-D-glucopyranosyl ester | - | $C_{50}H_{80}O_{28}$ | 1129 |
| Rebaudioside Q3 | SvG5 | Glcβ1- | Glcα(1-4) Glcβ(1-3) [Glcβ(1-2)]Glcβ1- | 13-[(4-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-2-O-β-D-glucopyranosyl- β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl ester | - | $C_{50}H_{80}O_{28}$ | 1129 |
| Rebaudioside *M* | SvG6 | Glcβ(1-2)[Glcβ(1-3)]Glcβ1- | Glcβ(1-2)[Glcβ(1-3)]Glcβ1- | 13-[(*O*-β- D-glucopyranosyl-(1,2)-*O*-[ β- D-glucopyranosyl-(1,3)]-β- D-glucopyranosyl)oxy]-kaur-16-en-18-oic acid (4')-*O*-β- D-glucopyranosyl-(1,2)-*O*-[β- D-glucopyranosyl-(1,3)]-β- D-glucopyranosyl ester | 1220616-44-3 | $C_{56}H_{90}O_{33}$ | 1291 |

| Common Name | Trivial Name | $R_1$ | $R_2$ | Chemical Name | CAS Number | Chemical Formula | Formula Weight |
|---|---|---|---|---|---|---|---|
| Related SvGn#1 | | - | - | - | - | $C_{21}H_{30}O_{11}$ | 458 |
| Related SvGn#2 | | - | - | - | - | $C_{40}H_{70}O_{24}$ | 982 |
| Related SvGn#3 | | - | - | - | - | $C_{32}H_{52}O_{15}$ | 676 |
| Related SvGn#4 | | - | - | - | - | $C_{50}H_{80}O_{28}$ | 1129 |
| Related SvGn#5 | | - | - | - | - | $C_{40}H_{70}O_{24}$ | 982 |
| **Group 2: Steviol + Rhamnose + Glucose (SvR1Gn)** | | | | | | | |
| Dulcoside *A* | SvR1G2 | Glcβ1- | Rhaα(1-2)Glcβ1- | 13-[(2-O-α–L-rhamnopyranosyl-β–D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester | 64432-06-0 | $C_{38}H_{60}O_{17}$ | 789 |
| Dulcoside C | SvR1G2 | H | Rhaα(1-2)[Glcβ(1-3)] Glcβ1- | 13-[(2-O-β-D-rhamnopyranosyl-3-β-D-glucopyranosyl- β-D-glucopyranosyl -oxy]kaur-16-en-18-oic acid | | $C_{38}H_{60}O_{17}$ | 789 |
| Rebaudioside *C* | SvR1G3 | Glcβ1- | Rhaα(1-2)[Glcβ(1-3)]Glcβ1- | 13-[(2-O-α–L-rhamnopyranosyl-3-O-β–D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester | 63550-99-2 | $C_{44}H_{70}O_{22}$ | 951 |
| Rebaudioside C2 | SvR1G3 | Rhaα(1-2)Glcβ1 | Glcβ(1-2)Glcβ1- | 13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-rhamnopyranosyl- β-D-glucopyranosyl ester | - | $C_{44}H_{70}O_{22}$ | 951 |
| Rebaudioside *N* | SvR1G5 | Rhaα(1-2)[Glcβ(1-3)]Glcβ1- | Glcβ(1-2)[Glcβ(1-3)]Glcβ1- | 13-[(2-*O*-β- D-glucopyranosyl-(1,2)-*O*-[β- D-glucopyranosyl-(1,3)]-β- D-glucopyranosyl)oxy]-kaur-16-en-18-oic acid (4')-*O*-2-deoxy-L-rhmanopyranosyl-3-*O*-β- D-glucopyranosyl-β- D-glucopyranosyl ester | 1220616-46-5 | $C_{56}H_{90}O_{32}$ | 1274 |

| Common Name | Trivial Name | $R_1$ | $R_2$ | Chemical Name | CAS Number | Chemical Formula | Formula Weight |
|---|---|---|---|---|---|---|---|
| Rebaudioside *O* | SvR1G6 | Glcβ(1-3)Rhaα(1-2)[Glcβ(1-3)]Glcβ1- | Glcβ(1-2)[Glcβ(1-3)]Glcβ1- | 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] ent-kaur-16-en-19-oic acid-[(2-O-(3-O-β-D-glucopyranosyl-α-L-rhamnopyranosyl)-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl) ester] | 1220616-48-7 | $C_{62}H_{100}O_{37}$ | 1436 |
| Rebaudioside *O2* | SvR1G6 | Glcβ(1-4*)Rhaα(1-2)[Glcβ(1-3)]Glcβ1- | Glcβ(1-2)[Glcβ(1-3)]Glcβ1- | 13-[(*O*-β-D-glucopyranosyl-(1,2)-*O*-[β-D-glucopyranosyl-(1,3)]-β-D-glucopyranosyl)oxy]-kaur-16-en-18-oic acid (4')-*O*-β-D-glucopyranosyl-(1,4)-*O*-6- deoxy-L-rhmnopyranosyl-(1,2)-*O*-[β-D-glucopyranosyl-(1,3)]-β-D-glucopyranosyl ester | - | $C_{62}H_{100}O_{37}$ | 1436 |
| Rebaudioside K | SvR1G4 | Glcβ(1-2)Glcβ1- | Rhaα(1-2)[Glcβ(1-3)]Glcβ1- | 13-[(2-*O*-β-D-rhamnopyranosyl-3-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester | 1220616-40-9 | $C_{50}H_{80}O_{27}$ | 1112 |
| Rebaudioside *S* | SvR1G3 | Rhaα(1-2)Glcβ1- | Glcα (1-2)Glcβ1- | 13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, O-2-deoxy-L-rhamnopyranosyl β-D-glucopyranosyl ester | 1931085-11-8 | $C_{44}H_{70}O_{22}$ | 951 |
| Rebaudioside *K2* | SvR1G4 | Glcβ(1-6)Glcβ1- | Rhaα(1-2)[Glcβ(1-3)]Glcβ1- | 13-[(2-*O*-β-D-rhamnopyranosyl-3-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 6-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester | - | $C_{50}H_{80}O_{27}$ | 1112 |
| Rebaudioside *H* | SvR1G4 | Glcβ1- | Glcβ(1-3)Rhaα(1-2)[Glcβ(1-3)]Glcβ1- | 13-[(3-*O*-β-D-glucopyranosyl-2-O-β-D-rhamnopyranosyl-3-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester | 1220616-36-3 | $C_{50}H_{80}O_{27}$ | 1112 |

| Common Name | Trivial Name | $R_1$ | $R_2$ | Chemical Name | CAS Number | Chemical Formula | Formula Weight |
|---|---|---|---|---|---|---|---|
| Rebaudioside *J* | SvR1G4 | Rhaα(1-2)Glcβ1- | Glcβ(1-2)[Glcβ(1-3)]Glcβ1- | 13-[(2-*O*-β-D-glucopyranosyl-3-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]-kaur-16-en-18-oic acid, 2-O-6- deoxy-L-rhmnopyranosyl-β-D-glucopyranosyl ester | 1313049-59-0 | $C_{50}H_{80}O_{27}$ | 1112 |
| **Group 3: Steviol + Xylose + Glucose (SvX1Gn)** | | | | | | | |
| Stevioside F | SvX1G2 | Glcβ1- | Xylβ(1-2)Glcβ1- | 13-[(2-O-β-D-xylopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester | - | $C_{37}H_{59}O_{17}$ | 775 |
| Rebaudioside *F* | SvX1G3 | Glcβ1- | Xylβ(1-2)[Glcβ(1-3)]Glcβ1- | 13-[(2-*O*-β-D-xylopyranosyl-3-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester | 438045-89-7 | $C_{43}H_{68}O_{22}$ | 937 |
| Rebaudioside F2 | SvX1G3 | Glcβ1- | Glcβ (1-2)[ Xylβ (1-3)]Glcβ1- | 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-xylopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester | - | $C_{43}H_{68}O_{22}$ | 937 |
| Rebaudioside F3 | SvX1G3 | Xylβ(1-6) Glcβ1- | Glcβ(1-2)Glcβ1- | 13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 6-O-β-D-xylopyranosyl-β-D-glucopyranosyl ester | - | $C_{43}H_{68}O_{22}$ | 937 |
| Rebaudioside R | SvX1G3 | Glcβ1- | Glcβ(1-2)[Glcβ1-3] Xylβ1 | 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-xylopyranosyl-3)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester | 1931083-53-2 | $C_{43}H_{68}O_{22}$ | 937 |
| Rebaudioside *U2* | SvX1G4 | Xylβ(1-2*)[Glcβ(1-3)]Glcβ1- | Glcβ(1-2)Glcβ1- | 13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-D-xylopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester | - | $C_{50}H_{82}O_{26}$ | 1099 |

| Common Name | Trivial Name | $R_1$ | $R_2$ | Chemical Name | CAS Number | Chemical Formula | Formula Weight |
|---|---|---|---|---|---|---|---|
| Rebaudioside *T* | SvX1G4 | Xylβ(1-2)Glcβ1- | Glcβ(1-2)[Glcβ(1-3)]Glcβ1- | 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-D-xylopyranosyl-β-D-glucopyranosyl ester | | $C_{50}H_{82}O_{26}$ | 1099 |
| Rebaudioside *V2* | SvX1G5 | Xylβ (1-2)[Glcβ(1-3)]Glcβ1- | Glcβ(1-2)[Glcβ(1-3)]Glcβ1- | 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl- β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-D-xylopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester | - | $C_{56}H_{92}O_{31}$ | 1261 |
| Rebaudioside *V* | SvX1G5 | Glcβ(1-2)[Glcβ(1-3)]Glcβ1- | Xylβ(1-2*)[Glcβ(1-3)]Glcβ1- | 13-[(2-O-β-D-xylopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester | - | $C_{56}H_{92}O_{31}$ | 1261 |
| **Group 4: Steviol + Arabinose + Glucose (SvA1Gn)** | | | | | | | |
| Rebaudioside U | SvA1G4 | Araα(1-2*)Glcβ1 | Glcβ(1-2)[Glcβ(1-3)]Glcβ1- | 13-[(2-O- β -D-glucopyranosyl-3-O β -D-glucopyranosyl- β-D-glucopyranosyl) oxy]ent-kaur-16-en-19-oic acid-(6-O- α--L-arabinopyranosyl- β -D-glucopyranosyl) ester | | $C_{50}H_{82}O_{26}$ | 1098 |
| Rebaudioside W | SvA1G4 | Glcβ(1-2)[Araβ(1-3*)]Glcβ1 | Glcβ(1-2)Glcβ1- | 13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-Dglucopyranosyl-3-O-β-D-arabinopyranosyl-β-D-glucopyranosyl ester | - | $C_{50}H_{82}O_{26}$ | 1098 |

| Common Name | Trivial Name | $R_1$ | $R_2$ | Chemical Name | CAS Number | Chemical Formula | Formula Weight |
|---|---|---|---|---|---|---|---|
| Rebaudioside *W2* | SvA1G4 | Araβ(1-2*)Glcβ1 | Glcβ(1-2)[Glcβ(1-3)]Glcβ1- | 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl- β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-D-arabinopyranosyl-β-D-glucopyranosyl ester | - | $C_{50}H_{82}O_{26}$ | 1098 |
| Rebaudioside *W3* | SvA1G4 | Araβ(1-6)Glcβ1- | Glcβ(1-2)[Glcβ(1-3)]Glcβ1- | 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl- β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 6-O-β-D-arabinopyranosyl-β-D-glucopyranosyl ester | - | $C_{50}H_{82}O_{26}$ | 1098 |
| Rebaudioside *Y* | SvA1G5 | Glcβ(1-2)[Araβ(1-3*)]Glcβ1 | Glcβ(1-2)[Glcβ(1-3)]Glcβ1- | 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl- β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl-3-O-β-D-arabinopyranosyl-β-D-glucopyranosyl ester | - | $C_{56}H_{92}O_{31}$ | 1260 |
| **Group 5: Steviol + Galactose + Glucose (SvGa1Gn)** | | | | | | | |
| Rebaudioside *T1* | SvGa1G4 | Galβ(1-2*)Glcβ1 | Glcβ(1-2)[Glcβ(1-3)]Glcβ1- | 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl- β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-D-galactopyranosyl-β-D-glucopyranosyl ester | - | $C_{50}H_{80}O_{28}$ | 1128 |
| **Group 6: Steviol + Fructose + Glucose (SvFruGn)** | | | | | | | |
| Rebaudioside *A3* | SbF1G3 | Glcβ1- | Glcβ(1-2)[Fruβ(1-3)]Glcβ1- | 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-fructofuranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester | - | $C_{44}H_{70}O_{22}$ | 951 |

| Common Name | Trivial Name | $R_1$ | $R_2$ | Chemical Name | CAS Number | Chemical Formula | Formula Weight |
|---|---|---|---|---|---|---|---|
| **Group 7: Steviol + -de-oxy glucose + Glucose (SvdG1Gn)** | | | | | | | |
| Stevioside D | SvDg1G2 | Glcβ1- | 6-deoxy Glcβ(1-2)Glcβ1- | 13-[(2-O-β-D-6-deoxyglucopyranosyl-β-D-glucopyranosy)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester | - | $C_{38}H_{60}O_{17}$ | 789 |
| Stevioside E | SvDg1G3 | Glcβ1- | 6-deoxy Glcβ(1-2)[Glcβ(1-3)] Glcβ1- | 13-[(2-O-β-D-6-deoxyglucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester | - | $C_{44}H_{70}O_{22}$ | 951 |
| Stevioside E2 | SvDg1G3 | 6-deoxy Glcβ1- | Glcβ(1-2)[Glcβ(1-3)] Glcβ1- | 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl- β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-6-deoxyglucopyranosyl-ester | - | $C_{44}H_{70}O_{22}$ | 951 |

Steviol (R1 = R2 = H) is the aglycone of the steviol glycosides.

Glc, Rha, Fru, deoxyGlc, Gal, Ara and Xyl represent, respectively, glucose, rhamnose, fructose, deoxyglucose, glactose, arabinose and xylose sugar moieties.

Note: This list is not exhaustive. More steviol glycosides may have been identified in the plant of *Stevia rebaudiana* Bertoni, in the literature.

## *3. Description*

Commercial steviol glycoside preparations are white to light yellow powders that are freely soluble in ethanol and water (50:50) mixtures. The powders can be odourless or have a slight characteristic odour. Aqueous solutions are 200 to 300 times sweeter than sucrose.

## *4. Manufacture of steviol glycosides*

Steviol Glycosides from *Stevia rebaudiana* Bertoni is obtained from the leaves of *Stevia rebaudiana* Bertoni. The leaves are extracted with hot water and the aqueous extract is passed through an adsorption resin to trap and concentrate the component steviol glycosides. The resin is washed with a solvent alcohol to release the glycosides and the product is recrystallized from methanol or aqueous ethanol. Ion exchange resins may be used in the purification process. The final product may be spray-dried. The Committee, at its 68th and 69th meetings reviewed information on additional purification steps included in the manufacturing process of steviol glycosides considered by the 63rd JECFA. These steps included further recrystallization and separation steps; and the introduction of ethanol as a new solvent.

Rebaudioside A from Genetically Modified *Yarrowia lipolytica* is obtained from the fermentation of a non-toxigenic non-pathogenic strain of *Yarrowia lipolytica* that is genetically modified with heterologous genes from multiple donor organisms to overexpress steviol glycosides. After removal of the biomass by solid-liquid separation and heat treatment, the process involves concentration of the steviol glycosides (e.g. by resin adsorption), followed by purification of the rebaudioside A by crystallization and drying. Ion exchange resins may be used in the purification process. The final product may be spray-dried.

## *5. Chemical characterization*

Composition of Steviol Glycosides from the leaves of *Stevia rebaudiana* Bertoni

In response to the call for data on "stevioside" for the 63rd 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 63rd 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 defined as the sum of rebaudioside A, stevioside, rebaudioside C and dulcoside A. Analytical data showed that most of the remaining 5% could be accounted for by saccharides other than those associated with the individual steviol glycosides.

In response to the Call for Data of the 84th JECFA, analysis results from three batches of a commercial steviol glycoside preparation were submitted by the sponsor. Compositional analysis was conducted using a representative sample following HPLC/UV methodology for the major steviol glycosides (90 – 95 % of total steviol glycosides) using commercially available reference standards (Table 2). Minor glycosides (present in small quantities and their total corresponds to about 5% of the total glycosides) are identified using mass spectrometry (using molecular mass ion and diagnostic fragment ions of steviol glycosides) and using in-house prepared standards. The data is presented in Table-3.

The total steviol glycosides in the samples ranged from 97.3 – 102 % which meets the requirement of ≤95% stipulated in the specifications. The major steviol glycosides ranged from 93.14 – 97.62 % and minor steviol glycosides ranged from 3.90 - 4.39 %. Beyond the variation in the number of steviol glycosides detected in each sample, the content of each

individual steviol glycosides also varied in the samples. It can be expected that steviol glycoside preparations following slightly differing manufacturing methods, and/or using *Stevia rebaudiana* Bertoni leaves from different origins (which may differ in their steviol glycoside composition due to soil, climatic and other conditions) as the starting material, may result in additional steviol glycosides.

Noting that the additive could be produced with high purity (> 95%) and that all the steviol glycosides hydrolyze upon ingestion to steviol, on which the ADI is based, the Committee recognized that the revised specifications would cover a range of steviol glycoside preparations.

Composition of Rebaudioside A from Multiple Gene Donors Expressed in *Yarrowia lipolytica*

At the 82nd JECFA a new monograph for Rebaudioside A from Multiple Gene Donors Expressed in Yarrowia lipolytica was established. It is composed of at least 95% (on the anhydrous basis) of rebaudioside A (13-[(2-*O*-β-D-glucopyranosyl-3-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester, CAS 58543-16-1, chemical formula $C_{44}H_{70}O_{23}$) with minor amounts of other steviol glycosides.

**Table 2: Major Steviol Glycosides (for which reference standards are available) present in three commercial lots of a steviol glycoside preparation**

| Steviol Glycoside | Steviol glycoside (% w/w) | | |
|---|---|---|---|
| | **Lot A-1** | **Lot A-2** | **Lot A-3** |
| Rubusoside | ND | ND | ND |
| Steviolbioside | ND | ND | ND |
| Stevioside | 0.255 | 0.163 | 0.344 |
| Rebaudioside B | 0.077 | 0.104 | 0.358 |
| *Rebaudioside E* | 0.882 | 0.959 | 1.00 |
| Rebaudioside A | 3.39 | 2.94 | 4.3 |
| Rebaudioside D | 55.9 | 67.5 | 61.1 |
| *Rebaudioside M* | 27.4 | 17.3 | 24.1 |
| Dulcoside A | ND | ND | ND |
| Rebaudioside C | 0.013 | 0.027 | 0.008 |
| *Rebaudioside N* | 3.38 | 3.32 | 3.93 |
| *Rebaudioside O* | 1.84 | 1.82 | 2.42 |
| Rebaudioside F | ND | ND | ND |
| Total Major Steviol Glycosides | 93.14 | 94.13 | 97.62 |

ND = Not Detected

**Table 3: Minor steviol glycosides (using in-house prepared standards) present in three commercial lots of a steviol glycoside preparation**

| Steviol Glycoside | Steviol glycoside (% w/w) | | |
|---|---|---|---|
| | Lot A-1 | Lot A-2 | Lot A-3 |
| Rebaudioside T | 0.142 | 0.165 | 0.246 |
| Rebaudioside V | 0.090 | 0.088 | 0.054 |
| Rebaudioside J | 1.913 | 1.859 | 2.22 |
| Rebaudioside W<br>Rebaudioside U2 | 0.553 | 0.441 | 0.354 |
| Rebaudioside W2 | 0.256 | 0.247 | ND |
| Rebaudioside W3 | 0.408 | 0.379 | 0.250 |
| Rebaudioside I<br>Rebaudioside U | 0.173 | 0.128 | 0.649 |
| Rebaudioside K | 0.167 | 0.170 | 0.175 |
| Rebaudioside V2 | 0.379 | 0.245 | 0.357 |
| Rebaudioside H | 0.038 | 0.176 | 0.087 |
| Rebaudioside O2 | ND | ND | ND |
| Rebaudioside K | ND | ND | ND |
| Rebaudioside Y | ND | ND | ND |
| Total Minor steviol glycosides | 4.12 | 3.90 | 4.39 |

ND = Not Detected

*5.1 Possible impurities*

Products containing 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 63rd JECFA was increased to not more than 6% by the 68th JECFA and the maximum limit for residual methanol of 100 mg/kg also established by the 63rd JECFA was raised to 200 mg/kg. The 69th JECFA introduced a maximum limit for residual ethanol of 5000 mg/kg. Maximum limits of 1 mg/kg for both arsenic and lead were maintained. Possible microbiological contaminants may include total Coliforms, E. coli and salmonella. However, they were not detected in the products.

Rebaudioside A from Multiple Gene Donors Expressed in *Yarrowia lipolytica* will contain minor amounts of rebaudioside D, rebaudioside B, rebaudioside M, and stevioside and may contain minor amounts of steviol and kaurenoic acid which are intermediates in the biosynthetic pathway of steviol glycosides.

*5.2 Analytical methods*

IR, NMR, and Mass Spectrometry are used for the identification of steviol glycosides with different sugar orientations. Liquid chromatography with UV detection (HPLC-UV) was found to be suitable for their quantitation. The JECFA HPLC assay method was optimised and validated to achieve chromatographic separation and quantitation of 13 major steviol glycosides (stevioside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside E, rebaudioside F, rebaudioside M, rebaudioside N, rebaudioside O, dulcoside A, rubusoside and Steviolbioside) with commercially available reference standards. In addition, a liquid chromatography-mass spectrometry (LCMS) method has been validated to confirm the presence of minor steviol glycosides (present in low quantities) for which commercial reference standards are not currently available. After

confirming the presence of each minor steviol glycoside by analysing their respective mass spectrum (using molecular mass ion and diagnostic fragment ions); they are quantitated using molecular mass corrected peak areas (obtained in the UV chromatogram) from Rebaudioside A standard curve (constructed from UV peak areas). These methods allow quantitation of total steviol glycosides in the commercial products.

### *6. 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, table-top sweeteners, desserts, yoghurts, confectionaries, delicacies, and pickles.

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 63rd 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:

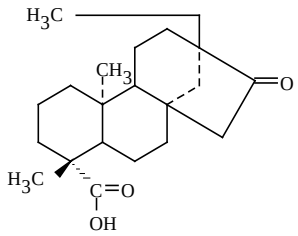


Figure 2. Isosteviol (C.A.S. no. 27975-19-5)

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-ß-D-glucopyranosyl-steviol, and 19% 13-O-ß-D-glucopyranosyl-steviol-ß-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 68th JECFA concluded that steviol glycosides is sufficiently thermally and hydrolytically stable for food use, including acidic beverages, under normal conditions of processing/storage.

Study results made available to JECFA for the 82nd meeting supported that the stability of steviol glycosides extract preparations established by JECFA at the 68th meeting can be extended to include steviol glycoside extract preparations containing higher levels of new glycosides added to the definition appearing in commercial products, mainly rebaudioside D and rebaudioside M.

## *7. Use levels in foods*

Food use-levels reported to the Committee at the 63rd, 68th and 69th meetings

| Food type | Reported maximum use-level (mg/kg) |
|---|---|
| Beverages (soft drinks, fruit drinks) | 600 |
| Desserts | 500 |
| Yogurt | 500 |
| Cold confectionery | 500 |
| Sauces | 1000 |
| Pickles | 1000 |
| Delicacies | 1000 |
| Sweet corn | 200 |
| Bread | 160 |
| Biscuits | 300 |

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

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