

SCHEDULE 2

Regulation 4(4)

Amendment to the Annex to Commission Regulation (EU) No 231/2012 for the authorisation of steviol glycosides from fermentation (*Yarrowia lipolytica*) (E 960b)

1. In the appropriate place, insert the following entry—

“E 960b STEVIOL GLYCOSIDES FROM FERMENTATION (*YARROWIA LIPOLYTICA*)

| Synonyms | | | | | | | | | | | | | |
|--------------------------|---|-------------------|---------|-------------------|----------------|---|------|----------------|---|------|----------------|---|------|
| Definition | <p>Steviol glycosides from <i>Yarrowia lipolytica</i> consist of a mixture predominantly composed of rebaudioside M, with some rebaudioside D, and smaller amounts of rebaudioside A and rebaudioside B. The manufacturing process comprises two main phases.</p> <p>The first phase involves fermentation of a non-toxicogenic non-pathogenic strain of <i>Yarrowia lipolytica</i> VRM that has been genetically modified with heterologous genes to overexpress steviol glycosides. Removal of biomass by solid-liquid separation and heat treatment is followed by concentration of the steviol glycosides.</p> <p>The second phase involves purification by employing ion-exchange chromatography, followed by recrystallisation of the steviol glycosides resulting in a final product containing not less than 95% of rebaudiosides M, D, A, and B.</p> <p>Viable cells or the DNA of <i>Yarrowia lipolytica</i> VRM must not be detected in the food additive.</p> | | | | | | | | | | | | |
| Chemical name | <p>Rebaudioside A: 13-[(2-<i>O</i>-β-D-glucopyranosyl-3-<i>O</i>-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester</p> <p>Rebaudioside B: 13-[(2-<i>O</i>-β-D-glucopyranosyl-3-<i>O</i>-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid</p> <p>Rebaudioside D: 13-[(2-<i>O</i>-β-D-glucopyranosyl-3-<i>O</i>-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-<i>O</i>-β-D-glucopyranosyl-β-D-glucopyranosyl ester</p> <p>Rebaudioside M: 13-[(2-<i>O</i>-β-D-glucopyranosyl-3-<i>O</i>-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-<i>O</i>-β-D-glucopyranosyl-3-<i>O</i>-β-D-glucopyranosyl-β-D-glucopyranosyl ester</p> | | | | | | | | | | | | |
| Molecular formula | <table border="1"> <thead> <tr> <th>Trivial name</th> <th>Formula</th> <th>Conversion factor</th> </tr> </thead> <tbody> <tr> <td>Rebaudioside A</td> <td>C₄₄ H₇₀ O₂₃</td> <td>0.33</td> </tr> <tr> <td>Rebaudioside B</td> <td>C₃₈ H₆₀ O₁₈</td> <td>0.40</td> </tr> <tr> <td>Rebaudioside D</td> <td>C₅₀ H₈₀ O₂₈</td> <td>0.29</td> </tr> </tbody> </table> | Trivial name | Formula | Conversion factor | Rebaudioside A | C ₄₄ H ₇₀ O ₂₃ | 0.33 | Rebaudioside B | C ₃₈ H ₆₀ O ₁₈ | 0.40 | Rebaudioside D | C ₅₀ H ₈₀ O ₂₈ | 0.29 |
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Status: This is the original version (as it was originally made). This item of legislation is currently only available in its original format.

| Synonyms | | | |
|---------------------------------|---|---|--------------------------|
| | Rebaudioside M | C ₅₆ H ₉₀ O ₃₃ | 0.25 |
| Molecular weight and CAS No | Trivial name | CAS Number | Molecular weight (g/mol) |
| | Rebaudioside A | 58543-16-1 | 967.01 |
| | Rebaudioside B | 58543-17-2 | 804.88 |
| | Rebaudioside D | 63279-13-0 | 1129.15 |
| | Rebaudioside M | 1220616-44-3 | 1291.29 |
| Assay | Not less than 95 % of rebaudioside A, rebaudioside B, rebaudioside D and rebaudioside M on the dried basis | | |
| Description | White to light yellow powder, approximately between 200 and 350 times sweeter than sucrose (at 5 % sucrose equivalency) | | |
| Identification | | | |
| Solubility | Freely soluble to slightly soluble in water | | |
| pH | Between 4.5 and 7.0 (1 in 100 solution) | | |
| hPurity | | | |
| Total ash | Not more than 1 % | | |
| Loss on drying | Not more than 6 % (105°C, 2h) | | |
| Residual solvent | Not more than 5000 mg/kg ethanol | | |
| Arsenic | Not more than 0.1 mg/kg | | |
| Lead | Not more than 0.1 mg/kg | | |
| Cadmium | Not more than 0.01 mg/kg | | |
| Mercury | Not more than 0.05 mg/kg | | |
| Residual protein | Not more than 20 mg/kg | | |
| Microbiological criteria | | | |
| Total (aerobic) plate count | Not more than 1000 CFU/g | | |
| Yeast | Not more than 100 CFU/g | | |
| Moulds | Not more than 100 CFU/g | | |
| <i>Escherichia coli</i> | Negative in 1g | | |
| <i>Salmonella</i> spp. | Negative in 25g". | | |