

## SCHEDULE 2

Regulation 3(3)

## Amendment of the Annex to Commission Regulation (EU) No. 231/2012

**“E 960c REBAUDIOSIDE M PRODUCED VIA ENZYME  
MODIFICATION OF STEVIOL GLYCOSIDES FROM STEVIA****Synonyms****Definition**

Rebaudioside M is a steviol glycoside composed predominantly of rebaudioside M with minor amounts of other steviol glycosides such as rebaudioside A, rebaudioside B, rebaudioside D, rebaudioside I, and stevioside.

Rebaudioside M is obtained via enzymatic bioconversion of purified steviol glycoside leaf extracts (95% steviol glycosides) of the *Stevia rebaudiana* Bertoni plant using UDP-glucosyltransferase and sucrose synthase enzymes produced by the genetically modified yeasts *K. phaffii* (formerly known as *Pichia pastoris*) UGT-a and *K. phaffii* UGT-b that facilitate the transfer of glucose from sucrose and UDP-glucose to steviol glycosides via glycosidic bonds.

After removal of the enzymes by solid-liquid separation and heat treatment, the purification involves concentration of the rebaudioside M by resin adsorption, followed by recrystallisation of rebaudioside M resulting in a final product containing not less than 95 % of rebaudioside M. Viable cells or the DNA of the yeasts *K. phaffii* UGT-a or *K. phaffii* UGT-b must not be detected in the food additive.

**Chemical name** Rebaudioside M: 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-glucopyranosyl-β-D-glucopyranosyl ester

<b>Molecular formula</b>	<b>Trivial name</b>	<b>Formula</b>	<b>Conversion factor</b>
	Rebaudioside M	C <sub>56</sub> H <sub>90</sub> O <sub>33</sub>	0.25

<b>Molecular weight and CAS No</b>	<b>Trivial name</b>	<b>CAS Number</b>	<b>Molecular weight (g/mol)</b>
	Rebaudioside M	1220616-44-3	1291.29

**Assay** Not less than 95 % 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)

**Purity**

**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

**Status:** This is the original version (as it was originally made). This item of legislation is currently only available in its original format.

<b>Arsenic</b>	Not more than 0.015 mg/kg
<b>Lead</b>	Not more than 0.2 mg/kg
<b>Cadmium</b>	Not more than 0.015 mg/kg
<b>Mercury</b>	Not more than 0.07 mg/kg
<b>Residual protein</b>	Not more than 5 mg/kg
<b>Particle size</b>	Not less than 74 µm (using a mesh #200 sieve with a particle size limit of 74 µm)”

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