SCHEDULE 1 Regulation 3(4)

Insertion of entry in the Annex to Commission Regulation (EU) 231/2012 for E 960b for Steviol Glycosides from Fermentation (*Yarrowia Lipolytica)*

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

Synonyms

Definition

Steviol glycosides from *Yarrowia lipolytica* 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.

The first phase involves fermentation of a non-toxigenic non-pathogenic strain of *Yarrowia. lipolytica* 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.

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.

Viable cells or the DNA of *Yarrowia Lipolytica* VRM must not be detected in the food additive.

Chemical name

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

Rebaudioside B: 13-[(2-*O*-β–D-glucopyranosyl-3-*O*-β– D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid

Rebaudioside D: 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

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

Molecular formula

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
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 M, rebaudioside D, rebaudioside A, and rebaudioside B 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		
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		
Escherichia coli	Negative in 1g		
***Salmonella* spp.**	Negative in 25g”		