Council of the European Union

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COVER NOTE

From:	Secretary-General of the European Commission, signed by Ms Martine DEPREZ, Director
date of receipt:	13 January 2025
To:	Ms Thérèse BLANCHET, Secretary-General of the Council of the European Union
No. Cion doc.:	D101884/02 ANNEXES 1 to 2
Subject:	ANNEXES to the COMMISSION REGULATION (EU) .../... amending Regulation (EC) No 1333/2008 of the European Parliament and the Council and Commission Regulation (EU) No 231/2012 as regards the use of Steviol glycosides produced by fermentation using *Yarrowia lipolytica*

Delegations will find attached document D101884/02 ANNEXES 1 to 2.

Encl.: D101884/02 ANNEXES 1 to 2

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PLAN/2024/1358 Rev1 ANNEX
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[…](2024) XXX draft

ANNEXES 1 to 2

ANNEXES

to the

COMMISSION REGULATION (EU) .../...

amending Regulation (EC) No 1333/2008 of the European Parliament and the Council and Commission Regulation (EU) No 231/2012 as regards the use of Steviol glycosides produced by fermentation using *Yarrowia lipolytica*

ANNEX I

Annex II to Regulation (EC) No 1333/2008 is amended as follows:

(a) in point 2 (Sweeteners) of Part B, the following entry is inserted after the entry for E 960a:

‘E 960b	Steviol glycosides from fermentation’

;

(b) in point (5) (Other additives that may be regulated combined) of Part C, point (v) is replaced by the following:

‘(v) E 960a – 960d: Steviol glycosides

E-number	Name
E 960a	Steviol glycosides from stevia
E 960b	Steviol glycosides from fermentation
E 960c	Enzymatically produced steviol glycosides
E 960d	Glucosylated steviol glycosides’

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ANNEX II

In the Annex to Regulation (EU) No 231/2012 the following entry is inserted after the entry for food additive E 960a:

'E 960b(i) Rebaudioside M from fermentation produced by *Yarrowia lipolytica*

Synonyms			
Definition	Rebaudioside M from fermentation produced by *Yarrowia lipolytica* consist of a mixture of steviol glycosides composed of rebaudioside M as the main componant, 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 simple sugar source by a non-toxigenic non-pathogenic strain of *Yarrowia lipolytica* that has been genetically modified with heterologous genes to overexpress genes which are involved in the synthesis of steviol glycosides to result in the strain VRM (CBS 147477). 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 cristallisation of the steviol glycosides from ethanol, resulting in a final product containing not less than 95% of rebaudiosides M, D, A and B. Viable cells and DNA of *Yarrowia lipolytica* VRM shall 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	**Trivial name**	**CAS Number**	**Molecular weight (g/mol)**

CAS No	Rebaudioside A	58543-16-1	967,01
	Rebaudioside B	58543-17-2	804,88
	Rebaudioside D	63279-13-0	1 129,15
	Rebaudioside M	1220616-44-3	1 291,30
Assay	Not less than 95% of rebaudioside M, rebaudioside D, rebaudioside A and rebaudioside B, on the dried basis.		
Particle size	Not more than 0,5% of particles of less than 300 nm (by STEM method)		
Description	White to light yellow powder, approximately between 200 and 350 times sweeter than sucrose (at 5 % sucrose equivalency).		
Identification			
Solubility	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 5 000 mg/kg ethanol		
Kaurenoic acid	Not more than 0,3 mg/kg (measured by liquid chromatography- mass spectrometry detection)		
Arsenic	Not more than 0,01mg/kg		
Lead	Not more than 0,01 mg/kg		
Cadmium	Not more than 0,01 mg/kg		
Mercury	Not more than 0,05 mg/kg		
Microbiological criteria			
Total (aerobic) plate count	Not more than 1 000 CFU/g		
Yeast and moulds	Not more than 100 CFU/g		

Escherichia coli	Negative in 1 g
Salmonella spp.	Negative in 25 g
Residual protein	Not more than 20 mg/kg’

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Salmonella spp.	Absent in 25 g
Escherichia coli	