



2025/652

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COMMISSION REGULATION (EU) 2025/652

of 2 April 2025

**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***

(Text with EEA relevance)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives ⁽¹⁾, and in particular Article 10(3) and Article 14 thereof,

Having regard to Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings ⁽²⁾, and in particular Article 7(5) thereof,

Whereas:

- (1) Annex II to Regulation (EC) No 1333/2008 lays down a Union list of food additives approved for use in foods and their conditions of use.
- (2) Commission Regulation (EU) No 231/2012 ⁽³⁾ lays down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008.
- (3) Those lists may be updated in accordance with the common procedure referred to in Article 3(1) of Regulation (EC) No 1331/2008, either on the initiative of the Commission or following an application.
- (4) Steviol glycosides obtained by three different manufacturing processes, namely, steviol glycosides from Stevia (E 960a), enzymatically produced steviol glycosides (E 960c) and glucosylated steviol glycosides (E 960d) are authorised as food additives and defined by separate specifications. These additives are regulated combined as defined in Part C of Annex II of Regulation (EC) No 1333/2008 ('the group of steviol glycosides (E 960a-960d)') with maximum limits expressed as steviol equivalents.
- (5) In September 2021, an application was received for amending the specifications for the group of steviol glycosides (E 960a-960d) to include a new method for the production of steviol glycosides, namely, of rebaudioside M produced from fermentation by *Yarrowia lipolytica*. Rebaudioside M from fermentation produced by *Yarrowia lipolytica* consists of not less than 95 % rebaudioside M, D, A and B and is obtained by fermentation of a source of simple sugar, using the genetically modified strain VRM of *Yarrowia lipolytica*. It is intended to be used under the same conditions as those already authorised for the group of steviol glycosides (E 960a-960d). The application was made available to the Member States pursuant to Article 4 of Regulation (EC) No 1331/2008.
- (6) On 24 October 2023, the European Food Safety Authority ('the Authority') adopted its opinion on the evaluation of the safety of rebaudioside M produced by fermentation using *Yarrowia lipolytica* ⁽⁴⁾. In its opinion, the Authority concluded that there is no safety concern for rebaudioside M, produced by fermentation using *Yarrowia lipolytica* to be used as a food additive for the proposed uses and at the proposed use levels, taking into account the existing ADI

⁽¹⁾ OJ L 354, 31.12.2008, p. 16, ELI: <http://data.europa.eu/eli/reg/2008/1333/oj>.

⁽²⁾ OJ L 354, 31.12.2008, p. 1, ELI: <http://data.europa.eu/eli/reg/2008/1331/oj>.

⁽³⁾ Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council (OJ L 83, 22.3.2012, p. 1, ELI: <http://data.europa.eu/eli/reg/2012/231/oj>).

⁽⁴⁾ EFSA Journal 2023; 21:e8387.

of 4 mg/kg bw per day (expressed as steviol equivalents). The Authority considered that this production method may result in impurities different from those that may be present in the other steviol glycosides, produced in accordance with the applicable respective specifications for E 960a, E 960c and E 960d. Therefore, the Authority considered that separate specifications are needed for the food additive produced via the manufacturing process described in the current application.

- (7) It is therefore appropriate to authorise the use for the proposed uses and at the proposed use levels of rebaudioside M produced by fermentation using *Yarrowia lipolytica*.
- (8) Taking into account the International Numbering System for food additives of the Codex Alimentarius, it is appropriate to authorise the food additive under the name 'Steviol glycosides from fermentation' (E 960b).
- (9) As the food additive steviol glycosides from fermentation (E 960b) may be used combined with additives from the group of steviol glycosides (E 960a–960d) in the food categories where those additives are currently authorised and at the same maximum levels as them, it should be added to the group of steviol glycosides (E 960a–960d) in Part C of Annex II to Regulation (EC) No 1333/2008.
- (10) The specifications for 'Steviol glycosides from fermentation' should be included in Regulation (EU) No 231/2012 in parallel with its inclusion in the Union list of food additives laid down in Annex II to Regulation (EC) No 1333/2008. Taking into account that other methods of production for steviol glycosides from fermentation (E 960b) could be authorised in the future, it is appropriate to include the specification as 'E 960b(i) rebaudioside M from fermentation produced by *Yarrowia lipolytica*'.
- (11) Regulations (EC) No 1333/2008 and (EU) No 231/2012 should therefore be amended accordingly.
- (12) The measures provided for in this Regulation are in accordance with the opinion of the Standing Committee on Plants, Animals, Food and Feed,

HAS ADOPTED THIS REGULATION:

Article 1

Annex II to Regulation (EC) No 1333/2008 is amended in accordance with Annex I to this Regulation.

Article 2

The Annex to Regulation (EU) No 231/2012 is amended in accordance with Annex II to this Regulation.

Article 3

This Regulation shall enter into force on the twentieth day following that of its publication in the *Official Journal of the European Union*.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 2 April 2025.

For the Commission
The President
Ursula VON DER LEYEN

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'
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(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'

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 <i>Yarrowia lipolytica</i> consist of a mixture of steviol glycosides composed of rebaudioside M as the main component, 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-toxicogenic non-pathogenic strain of <i>Yarrowia lipolytica</i> that has been genetically modified with heterologous genes to over-express 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 crystallisation 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 <i>Yarrowia lipolytica</i> 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 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	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 0,01 mg/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
<i>Escherichia coli</i>	Negative in 1 g
<i>Salmonella</i> spp.	Negative in 25 g
Residual protein	Not more than 20 mg/kg'