II

(Non-legislative acts)

REGULATIONS

COMMISSION REGULATION (EU) 2022/1922

of 10 October 2022

amending the Annex to Regulation (EU) No 231/2012 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 as regards specifications for rebaudiosides M, D and AM produced via enzymatic conversion of purified stevia leaf extracts and the specifications for rebaudioside M produced via enzyme modification of steviol glycosides from Stevia (E 960c(i))

(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 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) 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.

(2) The specifications for food additives 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 from a Member State or an interested party.

(3) The currently authorised food additive 'enzymatically produced steviol glycosides' (E 960c) are included in the group 'steviol glycosides (E960a-E960c)' in Part C of Annex II to Regulation (EC) No 1333/2008.

(4) Regulation (EU) No 231/2012 provides that 'rebaudioside M produced via enzyme modification of steviol glycosides from Stevia' (E 960c(i)) is obtained via enzymatic bioconversion of purified steviol glycoside leaf extracts of the *Stevia rebaudiana* Bertoni plant, using UDP-glucosyltransferase and sucrose synthase enzymes produced by the genetically modified yeasts *K. phaffi* UGT-a and *K. phaffi* UGT-b.

([1]) OJ L 354, 31.12.2008, p. 16.
([2]) OJ L 354, 31.12.2008, p. 1.
([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).

(5) On 18 February 2019, an application was submitted to the Commission for the amendment of the specifications concerning the food additive steviol glycosides (E 960). The Commission made the application available to the Member States pursuant to Article 4 of Regulation (EC) No 1331/2008.

(6) On June 2021, the applicant revised its application and requested enzymatic conversion of highly purified rebaudioside A or stevioside from Stevia leaf extracts to, respectively, rebaudiosides M and D and rebaudioside AM, using enzymes produced by genetically modified microorganisms derived from strains of *E. coli* K-12, to be used as an alternative production process for E 960c.

(7) This newly suggested production process involves the enzymatic conversion of purified rebaudioside A or stevioside stevia leaf extract (≥ 95 % steviol glycosides) through a multistep enzymatic process with enzymes prepared at the first stage of the process. According to the different duration of the enzymatic reaction on the purified rebaudioside A and stevioside stevia leaf extract, three main mixtures with a high content of rebaudioside M, D and AM can be obtained. The resulting mixtures undergo a series of purification and isolation steps to produce the final rebaudioside M, D or AM (≥ 95 % steviol glycosides).

(8) The European Food Safety Authority ('the Authority') evaluated the safety of 'steviol glycosides preparations obtained by enzymatic bioconversion of highly purified rebaudioside A or stevioside stevia leaf extracts' and adopted its opinion on 22 June 2021 ([4]). The Authority considered that there is no safety concern for steviol glycosides with a high content of rebaudioside M, rebaudioside D and rebaudioside AM, when obtained by the process in question, to be used as food additives. The Authority considered that exposure to rebaudioside AM (expressed as steviol equivalent) will not be higher than the exposure to steviol glycosides (E 960a), if those would be replaced by rebaudioside AM. The Authority further concluded that the ADI of 4 mg/kg bw per day that applies to the 60 steviol glycosides listed in Appendix A to the opinion adopted on 24 March 2020 ([5]), expressed as steviol equivalent, also applies to the rebaudioside M, D and AM obtained by the enzymatic bioconversion in question.

(9) Therefore, specifications for rebaudioside M, D and AM produced via enzymatic conversion of purified rebaudioside A or stevioside stevia leaf extract should be set out in the Annex to Regulation (EU) No 231/2012.

(10) Furthermore, in order to ensure clarity, it is appropriate to align the current definition of the additive 'Rebaudioside M produced via enzyme modification of steviol glycosides from stevia' in the entry E 960c(i) of the Annex to Regulation (EU) No 231/2012 with the wording of the conclusion of the Authority on the safety of its manufacturing process as regards the absence of viable cells and of residual DNA of the yeasts *K. phaffii* UGT-a and *K. phaffii* UGT-b in the food additive.

(11) Regulation (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

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

Article 2

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

([4]) *EFSA Journal* 2021;19(7):6691, 22 pp.
([5]) *EFSA Journal* 2020;18 (4):6106, 32 pp.

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

Done at Brussels, 10 October 2022.

For the Commission
The President
Ursula VON DER LEYEN

ANNEX

The Annex to Regulation (EU) No 231/2012 is amended as follows:

(1) in the entry for E 960c(i) Rebaudioside M produced via enzyme modification of Steviol glycosides from stevia, in the row 'Definition' the last sentence is replaced by the following:

'Viable cells of the yeasts *K. phaffii* UGT-a and *K. phaffii* UGT-b and their DNA shall not be detected in the food additive.';

(2) the following entries are inserted after the entry for E 960c(i):

'E 960c(ii) REBAUDIOSIDE M PRODUCED VIA ENZYMATIC CONVERSION OF HIGHLY PURIFIED REBAUDIOSIDE A STEVIA LEAF EXTRACTS

Synonyms			
Definition	Rebaudioside M produced via enzymatic conversion of highly purified rebaudioside A stevia leaf extracts is a steviol glycoside composed predominantly of rebaudioside M with minor amounts of other steviol glycosides such as rebaudioside A and rebaudioside D. Rebaudioside M is produced via enzymatic conversion of highly purified steviol glycoside rebaudioside A extracts (95 % steviol glycosides) obtained from *Stevia rebaudiana* Bertoni plant using UDP-glucosyltransferase and sucrose synthase enzymes produced by the genetically modified strains of *E. coli* (pPM294, pFAF170 and pSK401) 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 the steviol glycosides resulting in a final product containing not less than 95 % of rebaudioside M. Viable cells of *E. coli* (pPM294, pFAF170 and pSK401) and their DNA shall 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		
Molecular formula	Trivial name	Formula	Conversion factor
	Rebaudioside M	$C_{56}H_{90}O_{33}$	0,25
Molecular weight and CAS No	Trivial name	CAS Number	Molecular weight (g/mol)
	Rebaudioside M	1220616-44-3	1 291,29
Assay	Not less than 95 % rebaudioside M on the dried basis.		
Description	White to light yellow powder, approximately between 150 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 5 000 mg/kg ethanol
Arsenic	Not more than 0,015 mg/kg
Lead	Not more than 0,2 mg/kg
Cadmium	Not more than 0,015 mg/kg
Mercury	Not more than 0,07 mg/kg
Residual protein	Not more than 5 mg/kg
Particle size	Not less than 74 μm [using a mesh #200 sieve with a particle size limit of 74 μm]

E 960c(iii) REBAUDIOSIDE D PRODUCED VIA ENZYMATIC CONVERSION OF HIGHLY PURIFIED REBAUDIOSIDE A STEVIA LEAF EXTRACTS

Synonyms			
Definition	Rebaudioside D produced via enzymatic conversion of highly purified rebaudioside A stevia leaf extracts is a steviol glycoside composed predominantly of rebaudioside D with minor amounts of other steviol glycosides such as rebaudioside A and rebaudioside M. Rebaudioside D is produced via enzymatic conversion of highly purified steviol glycoside rebaudioside A extracts (95 % steviol glycosides) obtained from *Stevia rebaudiana* Bertoni plant using UDP-glucosyltransferase and sucrose synthase enzymes produced by the genetically modified strains of *E. coli* (pPM294, pFAF170 and pSK401) 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 D by resin adsorption, followed by recrystallisation of the steviol glycosides resulting in a final product containing not less than 95 % of rebaudioside D and rebaudioside A. Viable cells of *E. coli* (pPM294, pFAF170 and pSK401) and their DNA shall not be detected in the food additive.		
Chemical name	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 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		
Molecular formula	Trivial name	Formula	Conversion factor
	Rebaudioside D	$C_{50}H_{80}O_{28}$	0,29
	Rebaudioside A	$C_{44}H_{70}O_{23}$	0,33
Molecular weight and CAS No	Trivial name	CAS Number	Molecular weight (g/mol)
	Rebaudioside D	63279-13-0	1 291,15
	Rebaudioside A	58543-16-1	967,01
Assay	Not less than 95 % rebaudiosides D and A on the dried basis.		
Description	White to light yellow powder, approximately between 150 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 5 000 mg/kg ethanol
Arsenic	Not more than 0,015 mg/kg
Lead	Not more than 0,2 mg/kg
Cadmium	Not more than 0,015 mg/kg
Mercury	Not more than 0,07 mg/kg
Residual protein	Not more than 5 mg/kg
Particle size	Not less than 74 μm [using a mesh #200 sieve with a particle size limit of 74 μm]

E 960c(iv) REBAUDIOSIDE AM PRODUCED VIA ENZYMATIC CONVERSION OF HIGHLY PURIFIED STEVIOSIDE STEVIA LEAF EXTRACTS

<table>
<tr><td>Synonyms</td><td colspan="3"></td></tr>
<tr><td>Definition</td><td colspan="3">Rebaudioside AM produced via enzymatic convertion of highly purified stevioside stevia leaf extracts is a steviol glycoside composed predominantly of rebaudioside AM with minor amounts of other steviol glycosides such as stevioside and rebaudioside E.
Rebaudioside AM is produced via enzymatic conversion of highly purified steviol glycoside stevioside extracts (95 % steviol glycosides) obtained from Stevia rebaudiana Bertoni plant using UDP-glucosyltransferase and sucrose synthase enzymes produced by the genetically modified strains of E. coli (pPM294, pFAF170 and pSK401) 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 AM by resin adsorption, followed by recrystallisation of the steviol glycosides resulting in a final product containing not less than 95 % of rebaudioside AM. Viable cells of E. coli (pPM294, pFAF170 and pSK401) and their DNA shall not be detected in the food additive.</td></tr>
<tr><td>Chemical name</td><td colspan="3">Rebaudioside AM: 13-[(2-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.</td></tr>
<tr><td rowspan="2">Molecular formula</td><td>Trivial name</td><td>Formula</td><td>Conversion factor</td></tr>
<tr><td>Rebaudioside AM</td><td>C_{50} H_{80} O_{28}</td><td>0,29</td></tr>
<tr><td rowspan="2">Molecular weight and CAS No</td><td>Trivial name</td><td>CAS Number</td><td>Molecular weight (g/mol)</td></tr>
<tr><td>Rebaudioside AM</td><td>2222580-26-7</td><td>1 291,15</td></tr>
<tr><td>Assay</td><td colspan="3">Not less than 95 % rebaudioside AM on the dried basis.</td></tr>
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Description	White to light yellow powder, approximately between 150 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 5 000 mg/kg ethanol
Arsenic	Not more than 0,015 mg/kg
Lead	Not more than 0,2 mg/kg
Cadmium	Not more than 0,015 mg/kg
Mercury	Not more than 0,07 mg/kg
Residual protein	Not more than 5 mg/kg
Particle size	Not less than 74 μm [using a mesh #200 sieve with a particle size limit of 74 μm]'