3 November 2023
268-23

Approval report – Application A1268

Steviol glycosides produced by bioconversion using new enzymes produced by GM *Escherichia coli*

Food Standards Australia New Zealand (FSANZ) has assessed an application made by Manus Bio Inc. to amend the Australia New Zealand Food Standards Code to permit three enzymes from genetically modified (GM) *Escherichia coli* strain K-12 as processing aids in the manufacture (by bioconversion) of steviol glycosides.

On 15 June 2023, FSANZ sought submissions on a draft variation and published an associated report. FSANZ received four submissions.

FSANZ approved the draft variation on 25 October 2023. The Food Ministers' Meeting[1] was notified of FSANZ's decision on 3 November 2023.

This Report is provided pursuant to paragraph 33(1)(b) of the *Food Standards Australia New Zealand Act 1991* (the FSANZ Act).

[1] Formerly referred to as the Australia and New Zealand Ministerial Forum on Food Regulation

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Supporting document

The following document which informed the assessment of this application is available on the FSANZ website:

SD1 Risk and technical assessment report (at Approval)

Executive summary

Manus Bio Inc. applied to Food Standards Australia New Zealand (FSANZ) to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of three new genetically modified (GM) enzymes, that are also protein engineered, for the bioconversion production method of two steviol glycosides, rebaudiosides M and I. The enzymes are:

1. Uridine triphosphate (UTP)-glucose-1-phosphate uridylyltransferase (EC 2.7.7.9) produced by GM *Escherichia* coli K-12, containing the gene for UTP-glucose-1-phosphate uridylyltransferase from *Bifidobacterium bifidum*
2. Uridine diphosphate (UDP)-glucosyltransferase produced by GM *Escherichia coli* K-12, containing the gene for UDP-glucosyltransferase from *Oryza sativa* (rice)
3. Sucrose synthase (EC 2.4.1.13) produced by GM *Escherichia coli* K-12, containing the gene for sucrose synthase from *Glycine max* (soybean).

Steviol glycosides are permitted in the Code to be added as intense sweeteners to specific foods via Schedule 15. There are current permissions for rebaudiosides M and I as permitted steviol glycosides via specific specifications within Schedule 3, but not for the applicant's form of these steviol glycosides.

FSANZ's risk assessment found the three enzymes are technologically justified for use as processing aids in the bioconversion production method of steviol glycosides. All three enzymes are used together in the production of the Applicant's rebaudioside M. Only enzymes 1 and 3 – as listed above – are used together in the production of the Applicant's rebaudioside I.

No residual protein or DNA of the microorganisms and enzymes remains in the purified steviol glycosides, and the purity complies with the relevant JECFA specifications for steviol glycosides.

The production organism *E. coli* strain K-12 has a long history of safe use. The derived GM production strains developed specifically to produce rebaudiosides M and I are neither pathogenic nor toxigenic and do not present a food safety risk. Analysis of the GM production strain confirmed the insertion and stability of the inserted genes.

The enzymes have a history of safe use for steviol glycoside production. For all three enzymes, the inserted genetic material is from a species with a long history of safe use. Recent bioinformatics searches were conducted by comparing the amino acid sequences of the three enzymes to those of known toxins and known allergens. No homologies of concern were identified in these searches.

Based on the reviewed data it was concluded that in the absence of any identifiable hazard, an Acceptable Daily Intake (ADI) 'not specified' was appropriate for all three enzymes.

The bioconversion production method of steviol glycosides is a well-known and assessed method that has permissions in international regulations, including Codex Alimentarius standards and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) specifications – (Framework for) Steviol Glycosides (being annex 3). The bioconversion method of production of steviol glycosides has also been assessed by FSANZ a number of times and the applicant's bioconversion method of production of steviol glycosides has been assessed as safe and suitable.

Following assessment and the preparation of a draft variation to the Code, FSANZ called for submissions regarding the draft variation on 15 June 2023. Four submissions were received

which raised issues that have been addressed in this report. Having regard to those submissions and for reasons set out in this report, FSANZ assessed that the draft variation proposed at the call for submissions needed to be amended.

For reasons set out in this report, FSANZ has approved an amended draft variation to the Code, amending Schedules 3 and 18. The effect of the approved draft variation (as amended) will be to permit the applicant's bioconversion method of producing the steviol glycosides: rebaudiosides I and M. This method uses the three protein engineered enzymes listed above as processing aids. The draft variation also permits the use of these three enzymes for that specific purpose, in accordance with the Code.

1 Introduction

1.1 The applicant

Manus Bio Inc. (Manus Bio) is a manufacturer of flavours, fragrances, food ingredients, cosmetics, vitamins, pharmaceuticals and agricultural chemicals. It uses fermentation technology to produce steviol glycosides, an intense sweetener food additive permitted to be added to various food products.

1.2 The application

Manus Bio requested permission for the use of three new genetically modified (GM) enzymes for the bioconversion production method of two steviol glycosides, rebaudioside M and rebaudioside I, in the Australia New Zealand Food Standards Code (the Code). These enzymes are not currently permitted in the Code for such use.

The enzymes are:

1. Uridine triphosphate (UTP)-glucose-1-phosphate uridylyltransferase (EC 2.7.7.9) produced by GM *Escherichia* coli K-12, containing the gene for UTP-glucose-1-phosphate uridylyltransferase from *Bifidobacterium bifidum*
2. Uridine diphosphate (UDP)-glucosyltransferase produced by GM *Escherichia coli* K-12, containing the gene for UDP-glucosyltransferase from *Oryza sativa* (rice)
3. Sucrose synthase (EC 2.4.1.13) produced by GM *Escherichia coli* K-12, containing the gene for sucrose synthase from *Glycine max* (soybean).

All three enzymes are used together in the production of the Applicant's rebaudioside M. Only enzymes 1 and 3 – as listed above – are used together in the production of the Applicant's rebaudioside I.

The applicant's method of production is variously called bioconversion, biotransformation, enzymatic conversion or 'enzyme modified', with 'bioconversion' being used in this report.

The Joint Expert Committee on Food Additives (JECFA) has recently published a Specifications Monograph – (Framework for) steviol glycosides (JECFA framework) within monograph 26 (2021) of the JECFA specifications (FAO 2021). This framework includes four methods of production as annexes. Annex 3 is titled 'Enzyme modified steviol glycosides' and sets out the method of production relevant to this application. The reason for the assessment of the application is that the applicant's steviol glycosides are not compliant with the JECFA framework as well as the Code.

Annex 3 of the JECFA framework contains the following definition of enzyme modified steviol glycosides: a process in which steviol glycosides that have been extracted from the leaves of *Stevia rebaudiana* Bertoni [*stevia* plant] undergo enzymatic conversion of major steviol glycosides to minor ones. In the applicant's case, the minor steviol glycosides are rebaudioside M and rebaudioside I.

FSANZ has already assessed a number of recent applications using the bioconversion method of manufacture - A1157, A1272, A1176 and A1183 (FSANZ 2018, FSANZ 2019a, FSANZ 2019b, FSANZ 2020 respectively) and the enzymes used for such manufacture are permitted in the Code.

1.3 The current standard

1.3.1 Australia and New Zealand standards

Australian and New Zealand food laws require food for sale to comply with relevant requirements in the Code. The requirements relevant to this application are summarised below.

1.3.1.1 Permitted use - processing aids

Enzymes used in food processing and manufacturing are considered processing aids as although they may be present in the final food, they no longer provide a technological purpose in the final food.

Paragraph 1.1.1—10(6)(c) provides that a food for sale must not have, as an ingredient or a component, a substance that is used as a processing aid, unless that substance's use as a processing aid is expressly permitted by the Code.

Section 1.1.2—13 provides that a substance 'used as a processing aid' in relation to a food is a substance used during the course of processing that meets all of the following conditions:

- it is used to perform a technological purpose during the course of processing
- it does not perform a technological purpose in the food for sale, and
- it is a substance listed in Schedule 18 or identified in section S16—2 as an additive permitted at GMP.

Standard 1.3.3 and Schedule 18 list the permitted processing aids. Enzymes of microbial origin listed in the table to subsection S18—4(5) are permitted for use as a processing aid to perform any technological purpose if the enzyme is derived from the corresponding source specified in the table. The table to subsection S18—9(3) lists those substances, including enzymes derived from particular sources, that are permitted to be used as processing aids for specific technological purposes in relation to:

- if a food is specified—that food; or
- if no food is specified—any food.

There are a number of enzymes listed within S18—9(3) permitted for the production of different steviol glycosides.

1.3.1.2 Permitted use - food additives

As noted, the application is to permit the use of the three enzymes that are used in the production of two specific steviol glycosides but is not seeking permission for steviol glycosides in general. However it does address the method of production of the applicant's specific steviol glycosides which are food additives (as intense sweeteners).

Paragraph 1.1.1—10(6)(a) provides that, unless expressly permitted by the Code, a food for sale cannot contain, as an ingredient or component, a substance that is used as a food additive.

Section 1.1.2—11 defines the expression 'used as a food additive'. Subsection 1.1.2—11(1) provides that a substance is 'used as a food additive' in relation to a food if both of the following conditions are met: the substance is added to the food to perform one or more technological functions listed in Schedule 14; and the substance is identified in subsection 1.1.2—11(2) – this includes (among other things) a substance identified in the table to

section S15—5 as a permitted food additive.

Section 1.3.1—3 details when substances are permitted to be used as food additives in food.

Schedule 14 lists the permitted technological purposes of food additives. The table in section S14—2 provides that use as an intense sweetener is a permitted purpose.

Schedule 15 lists the specific food additive permissions for different categories of foods in the table to section S15—5. 'Steviol glycosides' is listed in that table as a permitted food additive for various food categories with the International Numbering System (INS) number 960.

1.3.1.3 Food produced using gene technology

Paragraph 1.1.1—10(6)(g) requires that the presence of a food produced using gene technology as an ingredient or component in a food for sale must be expressly permitted by the Code. According to paragraph 1.5.2—3(b), permission in the Code for use as a food additive or processing aid also constitutes the permission required by paragraph 1.1.1—10(6)(g).

1.3.1.4 Identity and purity requirements

Paragraphs 1.1.1—15(1)(a) and (b) require substances used as food additives and processing aids respectively, to comply with any relevant identity and purity specifications listed in Schedule 3.

Subsection S3—2(1) of Schedule 3 incorporates by reference primary source specifications listed in the following: Joint FAO/WHO Expert Committee on Food Additives (JECFA) Combined Compendium of Food Additive Specifications (FAO 2021), explicitly FAO/WHO (2006); the United States Pharmacopeial Convention (FCC 2022) Food chemicals codex (13th edition); and the Commission Regulation (EU) No 231/2012. These include general identity and purity specifications for enzyme preparations used in food processing; and food additives.

Amendments resulting from Proposal P1061 – Code Maintenance Proposal 2023 (FSANZ 2023) updated the reference in the Code to the JECFA specifications to include monographs 25 and 26. As noted in section 1.2 of this report above, Monograph 26 includes steviol glycosides, being the (Framework for) Steviol Glycosides. This includes annex 3 which is the form of manufacture 'Enzyme modified' [bioconversion] steviol glycosides relevant to this application. The gazettal of amendments to the Code approved via P1061 occurred on 19 July 2023, which was before the final consideration of this application. As also noted earlier in the report the applicant's steviol glycosides do not comply with the JECFA framework.

Section S3—35 of Schedule 3 provides a specification for steviol glycosides produced by enzymatic conversion which is relevant to this application and so is required to be amended.

1.3.1.5 Labelling requirements

Paragraph 1.1.1—10(8) of the Code provides that food for sale must comply with all relevant labelling requirements in the Code.

Standard 1.2.1 sets out the labelling requirements for food for sale.

Standard 1.2.4 generally requires packaged food to be labelled with a statement of ingredients. Subsection 1.2.4—7(1) requires food additives to be declared in the statement of ingredients by one of the following ways: if the food additive can be classified into a class of

additives listed in Schedule 7—by referring to the relevant class name, followed in brackets by the name or code number of the food additive indicated in Schedule 8; otherwise—by referring to the name of the food additive as indicated in Schedule 8.

Schedule 7 lists the food additive class names that can be used in the statement of ingredients. Schedule 8 lists the names and code numbers of food additives that are to be used for labelling purposes.

Paragraphs 1.2.4—3(2)(d) and (e) exempt processing aids from the requirement to be declared in the statement of ingredients, unless other requirements apply.

Section 1.5.2—4 of the Code requires a food for sale that consists of a *genetically modified food*[2] (GM food) or has a GM food as an ingredient to be labelled as 'genetically modified', unless an exemption applies. The statement 'genetically modified' must be made in conjunction with the name of the GM food. If the GM food is used as a processing aid, this statement may be included in the statement of ingredients. In these circumstances, the requirements imposed by section 1.5.2—4 apply to foods for retail sale and to foods sold to a caterer in accordance with Standard 1.2.1.

1.4 International standards

1.4.1 International standards for processing aids

In developing food regulatory measures, Food Standards Australia New Zealand (FSANZ) must have regard to the promotion of consistency between domestic and international food standards. In terms of food safety, the relevant international standard setting body is the Codex Alimentarius Commission (Codex). There is no Codex Alimentarius 'general standard' for enzymes, however as noted above there are internationally recognised specifications for enzyme preparations established by JECFA and Food Chemicals Codex.

In addition, there is a Codex guideline, *Guidelines on Substances used as Processing Aids* (CAC/GL 75-2010), which sets out general principles for the safe use of substances used as processing aids, including that substances used as processing aids shall be used under conditions of GMP.

1.4.2 International standards for steviol glycosides

Steviol glycosides are approved for use in a number of other jurisdictions, including the European Union, Canada, South and North Asia, Asia Pacific, United States of America (USA), Central/South America, the Middle East and Africa (PureCircle Stevia Institute 2021). In the European Union, commercially available steviol glycoside products must comply with the specifications for steviol glycosides (INS number 960) adopted by the European Commission in 2012 and updated in 2016 (EC 2012, EC 2016).

1.4.2.1 Codex Alimentarius

Codex Alimentarius has a General Standard for Food Additives (GSFA, CXS 192-1995) that contains provisions for food additives in various food categories (Codex 2021a). The GSFA contains permissions for the addition of steviol glycosides (as steviol equivalents) to a wide variety of food categories up to maximum permitted levels. The GSFA includes four types of steviol glycosides, being:

[2] Section 1.5.2—4(5) defines ***genetically modified food*** to mean a '*food produced using gene technology that
a) contains novel DNA or novel protein; or
b) is listed in Section S26—3 as subject to the condition that its labelling must comply with this section' (*that being section 1.5.2—4*).

- INS 960a - Steviol glycosides from Stevia rebaudiana Bertoni (Steviol glycosides from Stevia)
- INS 960b - Steviol glycosides fermentation
- INS 960c - Enzymatically produced steviol glycosides
- INS 960d - Glucosylated steviol glycosides

Codex also has a guideline CXG 36-1989, Class Names and the International Numbering System (INS) for Food Additives (Codex 2021b) which lists the Codex names and numbering (INS) of food additives. It includes the names and INS numbers listed above that have been updated in the GSFA but there is an earlier entry which has not yet been removed. The report of the 52nd meeting of the Codex Committee on Food Additives (CCFA) in September 2021 endorsed these new INS names and number for the four different methods of production which was then adopted by the Codex Alimentarius Committee at its 44th meeting in 2021 and added into the GSFA and CXG 36-1989.

1.4.2.2 Joint FAO/WHO Expert Committee on Food Additives (JECFA)

As noted in section 1.2 JECFA Monograph 26 (91st meeting of February 2021) includes the (Framework for) Steviol Glycosides produced by four different methods. These are listed below with a short summary of the method of production:

Annex 1 ***Extraction from the leaves of*** **Stevia rebaudiana** ***Bertoni***
Annex 2 ***Steviol glycosides from fermentation***: a GM microorganism used to produce specific steviol glycosides
Annex 3 ***Enzyme modified steviol glycosides***: steviol glycosides extracted from the leaves (Annex 1) undergo bioconversion of major steviol glycosides to minor ones
Annex 4 ***Enzyme modified glycosylated steviol glycosides:*** steviol glycosides extracted from the leaves (Annex 1) undergo enzyme catalysed reactions to add glucose units to steviol glycosides.

1.4.2.3 USA

In the USA there have been over 50 GRAS (Generally Recognized as Safe) notifications relating to steviol glycosides submitted to the USA Food and Drug Administration (FDA) for review. GRN No. 1010 relates to the same production method and preparation of rebaudioside M as this application. GRN No. 1010 was submitted by the applicant in August 2021 and the US FDA responded with 'no questions' to the GRAS status[3]. Therefore, the applicant's rebaudioside M is considered GRAS for use as a general-purpose sweetener in foods in the US (USFDA 2022).

1.4.2.4 Canada

In Canada 'Steviol glycosides from *Stevia rebaudiana* Bertoni' are permitted in a variety of foods, provided they comply with the relevant international specifications for steviol glycosides (either JECFA or Food Chemicals Codex) and relevant conditions for use and requirements of the Food and Drug Act (Health Canada 2023).

1.4.2.5 European Union

Steviol glycosides preparations are permitted as food additives in a variety of different food

[3] 'No questions' response means the FDA does not question the basis for the notifier's GRAS conclusion (USFDA 2016).

categories (European Commission 2011) provided they comply with the European Commission specifications for steviol glycosides (European Commission 2016). The specifications have been updated so some do apply to steviol glycosides preparations produced by bioconversion.

1.5 Reasons for accepting application

The application was accepted for assessment because:

- it complied with the procedural requirements under subsection 22(2) of the *Food Standards Australia New Zealand Act 1991* (FSANZ Act)
- it related to a matter that warranted the variation of a food regulatory measure.

1.6 Procedure for assessment

The application was assessed under the General Procedure.

1.7 Decision

The draft variation as proposed following assessment was approved with amendments described below in section 2.1. The variation takes effect on gazettal. The approved draft variation, as amended, is at Attachment A.

The related explanatory statement is at Attachment B. An explanatory statement is required to accompany an instrument if it is lodged on the Federal Register of Legislation.

The draft variation on which submissions were sought is at Attachment C.

2 Summary of the findings

2.1 Summary of issues raised in submissions

FSANZ called for submissions on the draft variation to the Code from 15 June 2023 to 27 July 2023. Four submissions were received, one from a government agency and three from industry (Table 1). One late submission was received which supported one of the four submissions and did not raise any issues.

All submitters supported the approval of the application; being to approve amending the Code to permit the use of three protein engineered enzymes used as processing aids in the bioconversion method of production of the applicant's rebaudiosides M and I. However, several issues were raised in submissions from two submitters which have been summarised and addressed within Table 2. These submissions identified amendments that, in the submitters' opinion, should be made to the draft variation. The explanation for the amendments is provided in FSANZ's responses in Table 2.

The main topics raised in submissions can be summarised as:

- amendment of subsection S3—35(1) relating to the term 'prescribed rebaudioside'
- explicit provisions related to the production of the applicant's rebaudiosides M & I
- consequential changes to specifications, explicitly S3—35, via a future Code Revision proposal
- use of consistent language to identify enzymes (within S18—9(3))
- WTO notification to ensure transparency

- questions relating to the costs and benefits analysis.

As a result of considering the issues raised in submissions, FSANZ decided, in accordance with the FSANZ Act, to amend the draft variation as follows:

- remove the proposed change to subsection S3—35(1)
- include new specific and separate provisions for the production of the applicant's rebaudioside M, and rebaudioside I within subsection S3—35(2)
- specify in the table to S18—9(3) that:
  - the enzyme, uridine diphosphate (UDP)-glucosyltransferase, protein engineered variant, sourced from *Escherichia coli* K-12 containing the UDP-glucosyltransferase gene from *Oryza sativa* may be used as a processing aid in the conversion of purified stevia leaf extract to produce only rebaudioside M; and
  - the enzyme, uridine triphosphate (UTP)-glucose-1-phosphate uridylyltransferase, protein engineered variant, (EC 2.7.7.9) sourced from *Escherichia coli* K-12, containing the gene for UTP-glucose-1-phosphate uridylyltransferase from *Bifidobacterium bifidum*, 'expressing' is replaced by 'containing'.

More detail is provided in Table 2.

Table 1: List of submitters and summary position

Submitter	**Abbreviation**	**Submitter type**	**Support?**
Australian Food & Grocery Council	AFGC	Industry	Yes, with issues
Australian Beverages Council Limited	ABCL	Industry	Y, with issues
International Stevia Council	ISC	Industry	Yes
New Zealand Food Safety	NZFS	Government	Yes

Table 2: Summary of issues

Issue	Raised by	FSANZ response (including any amendments to drafting)
Currently under Schedule 3 – paragraph 35(2)(c), permissions for use of enzyme type and sources for the enzymatic conversion (bioconversion) production method for steviol glycosides are for prescribed rebaudiosides only as listed in subsection S3-35(1) i.e., rebaudiosides D, M and AM. With the proposed new wording (Attachment A to the Call for Submission report), it is not clear that the approval of the three new enzymes as per A1268 will only apply to rebaudiosides M and I. Users of these enzymes and/or ingredient (steviol glycoside) manufacturers may be confused with the proposed wording in paragraph S3-35(2)(c) as it implies that gene sources and enzymes are interchangeable across D, M, I and AM.	ABCL	FSANZ notes that the term 'prescribed rebaudiosides' is defined specifically for the purposes of S3—35(2)(c), and was added to the Code as a result of FSANZ's assessment of application A1176[4]. On that basis, FSANZ has re-evaluated the draft variation; assessed that the proposed amendment to S3—35(1) is not required for the purposes of application A1268, and decided to remove that amendment. Also S3—35(2)(c) cannot be amended as part of this application for the same reason.
Section S3—35(1) of Schedule 3 in the Code sets out the specifications for steviol glycosides produced by enzymatic conversion for the following prescribed rebaudiosides: (a) rebaudioside D; (b) rebaudioside M; and (c) rebaudioside AM Understand that permissions currently exist under subparagraph S3—35(2)(c) for A, D and AM only (interchangeable across all current gene sources and enzymes). With the introduction of three new gene sources and enzymes which are approved for M and I only, contend the lack of differentiation in wording under subparagraph (c) may prove confusing to infer that the gene sources and enzymes are not interchangeable across A, D, M, and I. Therefore recommends FSANZ consider including explicit wording which indicate specific permissions for rebaudiosides M and I.	AFCG	See the explanation above about why FSANZ is no longer proposing to amend subsection S3—35(1) or paragraph S3—35(2)(c). Instead, FSANZ's reconsideration of the draft variation recognises that new and separate amendments are required for the manufacture of the applicant's rebaudioside M and rebaudioside I. They have been kept separate like amendments in the variations prepared for other applications. FSANZ notes that in Schedule 3 of the Code, the specifications of different methods of production of steviol glycosides are specific for different applicants and specific methods of production, and are not general specifications. This is quite different to how the recent JECFA framework (monograph 26) operates as it is more general and does not relate to specific steviol glycosides. That is, there is not a one-to-one relationship between the specific method of production including the enzymes and source microorganism and specific steviol glycosides in the JECFA framework.

[4] https://www.foodstandards.gov.au/code/applications/Pages/A1176.aspx

Issue	Raised by	FSANZ response (including any amendments to drafting)
Notes via P1051 – Code Revision (2020), deletion of paragraph S3-35(2)(a) – preparation for steviol glycosides obtained from the leaves of the *Stevia rebaudiana* Bertoni plant (INS 960a). Notes this was due to the updates to paragraph S3—2(1)(b) to include reference to published JECFA specifications Monographs 22 (2018) and 23 (2019). There was also a subsequent change to the title of section S3—35 as a result. As per sub-section 1.3.1.4 in the A1268 consultation paper [Call for Submissions report], FSANZ notes P1061 Code Maintenance Proposal (2023) will include JECFA Monographs 25 and 26. Under Annex 3 of the JECFA Monograph 26 the method as described in S3—35(2)(c) enzymatic conversion method is listed. Seek clarity on the potential subsequent changes to S3—35 as a result of the inclusion of monographs 25 and 26 via P1061.	ABCL	The question of what (if any) further amendments will be made to Schedule 3 as a result of Proposal P1061 is out of scope and cannot be made via this application. However, FSANZ notes the amendment made to section S3—35 as a result of updates to the FAO JECFA Monographs in Proposal P1051. Now that the JECFA framework has been incorporated by reference into the Code because of amendments made by Proposal P1061, FSANZ may consider review of steviol glycoside specifications at some future time. Such a review would include consultation with stakeholders. What needs to be considered is whether it is appropriate or a priority to remove and consolidate steviol glycoside specifications within Schedule 3. FSANZ notes that some stakeholders, in particular earlier steviol glycosides applicants, may prefer these specifications to remain as listed in Schedule 3 to provide regulatory certainty. This is not something that could be addressed by a future Code Revision Proposal as it is not a minor matter.
Notes that as a result of Proposal P1051 – Code Revision (2020), reference to steviol glycoside preparations obtained from the leaves of the *Stevia rebaudiana* Bertoni plant in the then S3—35(2)(a) was removed as this method was captured in the updated Joint FAO/WHO Expert Committee on Food Additives (JECFA) Monographs 22 and 23 added to S3—2(1)(b) under the same Proposal. For the same reason stated above, recommends FSANZ consider whether the existing preparation methods currently listed in S3—35(2)(c) enzymatic conversion method (i – ii) could be removed in a future Code Maintenance Proposal assuming they are now captured by JECFA Monograph 26 Annex 3.	AFGC	Noted. However, this issue is out of scope for application A1268.

Issue	Raised by	FSANZ response (including any amendments to drafting)
Notes the draft variation (Attachment A in the Call for Submission report) inconsistently uses the terms 'containing' versus 'expressing' in the listings of the three new GM enzymes in subsection S18—9(3). Also note that in the consultation paper [Call for Submission report] and supporting document 1, the consistent term used is 'expressing' for all three enzymes.	ABCL	FSANZ agrees with the comment and notes the inconsistency in the use of 'containing' and 'expressing' in the table to subsection S18—9(3). FSANZ will change the terms used in the reports and also the approved draft variation to ensure the description of enzymes specific to this application is consistent with the majority of enzymes listed in Schedule 18 where 'containing' is used instead of 'expressing'. The relevant enzyme in item [2] of the draft variation to S18—9(3) will be amended as noted below: Uridine triphosphate (UTP)-glucose-1-phosphate uridylyltransferase, protein engineered variant, (EC 2.7.7.9) sourced from *Escherichia coli* K-12~~,~~ ~~expressing~~ **containing** the gene for UTP-glucose-1-phosphate uridylyltransferase from *Bifidobacterium bifidum.*
Notes in relation to the proposed listing in alphabetical order of the protein-engineered enzymes in the proposed draft variation, the usage of the word 'containing' for the first two enzymes versus the word 'expressing' for the third UTP enzyme. Seeks further clarity on the intent of using these different words and if they serve a specific purpose.	AFGC	See above response

Issue	Raised by	FSANZ response (including any amendments to drafting)
Notes FSANZ did not consider it necessary to submit a WTO notification under Australia and New Zealand's obligations to the WTO technical barriers to trade (TBT) or application of sanitary and phytosanitary (SPS) measures agreement. Recommends FSANZ consider the potential for trade implications with the approval of these three new GM enzymes in the production of steviol glycosides via bioconversion, given the differences in gene sources and enzymes between those listed in Codex Alimentarius (Codex) standards (JECFA specifications – Monograph 26) and set by FSANZ. Monitoring of changes to Codex standards and texts, including the Framework and agility to align national regulations with those amendments is imperative for trade facilitation. In this instance, while there may be harmonisation with production technologies, technical barriers to trade may arise between FSANZ and Codex without such equivalency in gene sources and enzymes listed.	ABCL	FSANZ notes that, as members of the World Trade Organization (WTO), Australia and New Zealand are obliged to notify WTO members where proposed mandatory regulatory measures are not substantially the same as existing international standards and the proposed measure may have a significant effect on trade. As stated in the assessment summary at the call for submissions, application A1268 is specifically about permitting three enzymes as processing aids to produce rebaudiosides by the bioconversion production method. There are JECFA specifications for steviol glycosides produced by different production methods, including bioconversion. This method of production is well known and regulated, and the enzymes are very comparable to already permitted forms and the enzymes perform the same function as those listed in the JECFA specification. FSANZ also notes that the proposed use of the enzymes as processing aids will be voluntary. Given the above, FSANZ considers that the proposed use of the enzymes as processing aids would facilitate trade in steviol glycosides and food containing steviol glycosides -not restrict it. That was the reason FSANZ did not notify WTO at the Call for Submission stage. However, notwithstanding the above, FSANZ has decided to notify WTO members as a matter of transparency.

Issue	Raised by	FSANZ response (including any amendments to drafting)
Notes reasons provided by FSANZ for not to submitting a WTO notification under Australia and New Zealand's obligations to the WTO technical barriers to trade (TBT) or application of sanitary and phytosanitary (SPS) measures agreement. Also notes that there are JECFA specifications (FAO and WHO 2021) for steviol glycosides produced by different production methods, including by bioconversion. This method of production is well known and regulated, and the enzymes are very comparable to already permitted forms in which the enzymes perform the same function as those listed in the JECFA specification. While the proposed amendments to the Code are 'trade facilitative', given the differences in gene sources and enzymes between those listed in JECFA specifications (Monograph 26) and what is proposed under this Application, recommends FSANZ consider submitting a WTO notification for transparency. This would support efforts to harmonise international and national standards.	AFGC	See above response.
Consideration of costs and benefits to industry: The overarching industry need for the approval of this variation is to harmonise with international regulations for market entry requirements. Secondly, it enables greater industry access to sufficient cost-effective supply of minor glycosides and approval for use at a country level.	ABCL	Any overall benefits to industry may come from lower costs and greater ability to produce rebaudiosides M and I. Given rebaudiosides M and I are already widely produced, overall benefits to industry as a whole would be small, uncertain and unquantifiable. Any such benefits are captured within the costs and benefits section, being section 2.5.1.1 of this report. Sufficient cost-effective supply is a subjective term. The costs and benefits section stated: *Benefits may include lower costs and higher efficiency of producing these rebaudiosides using this production method.*

2.2 Risk assessment

FSANZ conducted a risk assessment related to this application which is provided as SD1. The conclusion of this assessment is provided below.

The production organism *E. coli* K-12 strains has been genetically modified to produce the following enzymes used in the bioconversion production method of two steviol glycosides:

1. Uridine triphosphate (UTP)-glucose-1-phosphate uridylyltransferase (EC 2.7.7.9) produced by GM *Escherichia* coli K-12, containing the gene for UTP-glucose-1-phosphate uridylyltransferase from *Bifidobacterium bifidum*
2. Uridine diphosphate (UDP)-Glucosyltransferase produced by GM *Escherichia coli* K-12, containing the gene for UDP-glucosyltransferase from *Oryza sativa* (rice)
3. Sucrose synthase (EC 2.4.1.13) produced by GM *Escherichia coli* K-12, containing the gene for sucrose synthase from *Glycine max* (soybean).

The three enzymes are technologically justified for use in the production of steviol glycosides by the bioconversion method, consistent with the JECFA framework, and would function as processing aids for the purposes of the Code. The processing and purity steps undertaken ensure residual protein and residual DNA of microorganisms and enzymes is removed and not in the final purified steviol glycosides.

The bioconversion production method of steviol glycosides is a well-known and assessed method that has permissions in international regulations, including Codex Alimentarius standards and the JECFA framework (being annex 3). The bioconversion method of production of steviol glycosides has also been assessed by FSANZ a number of times and the applicant's bioconversion method of production of steviol glycosides has been assessed as safe and suitable.

All three enzymes are used together in the production of the applicant's rebaudioside M. Only enzymes 1 and 3 – as listed above – are used together in the production of the applicant's rebaudioside I.

The production strains derived from *E. coli* strain K-12 to produce rebaudiosides M and I are neither pathogenic nor toxigenic and do not present a food safety risk. Analysis of the GM production strains confirmed the insertion and stability of the inserted genes.

The enzymes have a history of safe use for steviol glycoside production. The production organism has a long history of safe use as an enzyme production organism. For all three enzymes, the inserted genetic material is from a species with a long history of safe use either as a supplement (*Bifidobacterium bifidum*) or as a food (rice, soybean). Recent bioinformatics searches were conducted by comparing the amino acid sequences of the three enzymes to those of known toxins and known allergens. No homologies of concern were identified in these searches.

Based on the reviewed data it was concluded that in the absence of any identifiable hazard, an Acceptable Daily Intake (ADI) 'not specified' is appropriate for all three enzymes.

2.3 Risk management

The bioconversion production method of steviol glycosides was comparable to methods already considered and permitted by FSANZ (see A1157, A1172, A1176 and A1183). As noted in the risk assessment conclusions FSANZ has concluded the applicant's bioconversion method of production is safe and suitable for the proposed purpose.

The risk management options available to FSANZ, after assessment, were to either reject the application or to prepare a draft variation to amend the Code to permit the applicant's three enzymes as processing aids to produce rebaudioside M and rebaudioside I, using the bioconversion production method.

No public health and safety concerns were identified during the assessment of the three enzymes. The enzymes were found to be technologically justified for their use to in the manufacture of the steviol glycosides. As the enzymes perform their technological function during the production process and not in the food for sale, the enzymes therefore function as processing aids for the purposes of the Code. Their use is also consistent with the JECFA framework.

FSANZ's assessment was that the use of the GM-derived enzymes to manufacture the steviol glycosides does not make the steviol glycosides GM foods. This was because the steviol glycosides are not themselves derived from an organism that has been modified using gene technology. The processing and purification steps undertaken ensure any residual protein or residual DNA from the microorganisms and enzymes is removed and not in the final purified steviol glycosides.

For the reasons set out in this report, FSANZ considers it was appropriate to prepare a draft variation to amend the Code. The approved amendments to the Code include listing the three enzymes as permitted processing aids within Schedule 18 for use in the production of the specific steviol glycosides.

In addition, the approved draft variation includes amendments to section S3—35 – Specifications for steviol glycosides produced by enzymatic conversion, which will list these new enzymes.

2.3.1 Labelling

2.3.1.1 Ingredient labelling

Under existing labelling requirements in the Code (unless the food for sale is exempt from the requirement for a statement of ingredients) the steviol glycosides will require declaration as a food additive in the statement of ingredients on the label of foods to which the steviol glycosides have been added (see section 1.3.1.5 above). These ingredient labelling requirements currently require steviol glycosides to be identified in the statement of ingredients using the prescribed class name 'sweetener' followed in brackets by either the food additive name 'steviol glycosides' or the International Numbering System (INS) code number 960 (as listed in Schedule 8).

As noted in section 1.4.3.1, the CCFA updated the INS numbers for steviol glycosides. FSANZ has not applied the updated numbering system as part of this application. This was to allow a more coordinated approach and efficient transition for the labelling of all steviol glycosides in the Code instead of an ad-hoc approach through various applications. For these reasons, FSANZ considered that the most appropriate INS number for labelling purposes, for all steviol glycosides at this stage, is 960. FSANZ will consider changes to this INS number in the future.

The FSANZ website provides information on the various production methods for steviol glycosides[5]. Consumers wanting to know the source of any particular steviol glycosides in

[5] For more information please see the following FSANZ webpage: Steviol glycosides (960) (intense sweetener) (stevia)

foods are advised that they may ask the manufacturer who should advise them accordingly.

In terms of the three enzymes used as processing aids, the generic exemption from listing processing aids in the statement of ingredients would apply (see section 1.3.1.5 above).

2.3.1.2 Labelling as 'genetically modified'

FSANZ's assessment was that Manus Bio's steviol glycosides are not GM foods and therefore would not require labelling as 'genetically modified'. This was in contrast to the enzymes used as processing aids for their manufacture, which are GM foods. However as noted in section 2.2 of this report, no residual protein or DNA of the microorganisms and enzymes would be present in the final purified steviol glycosides and therefore, would not be present in the food for sale. As such, the requirement to label the processing aids as 'genetically modified' would not apply to a food for sale that contains the steviol glycosides.

2.3.2 Risk management conclusion

The risk management conclusion is to prepare a draft variation permitting the applicant's bioconversion method of producing the steviol glycosides: rebaudiosides I and M, using three new protein-engineered enzymes sourced from genetically modified *Escherichia coli* as processing aids; and permitting the use of those enzymes for that specific purpose.

2.4 Risk communication

2.4.1 Consultation

Consultation is a key part of FSANZ's standards development process. FSANZ developed and applied a standard communication strategy to this application. All calls for submissions are notified via the Food Standards Notification Circular, media release, FSANZ's social media channels and Food Standards News.

The process by which FSANZ considers standards development matters is open, accountable, consultative and transparent. Public submissions were called to assist consideration of the draft variation to the Code. FSANZ acknowledges the time taken by individuals and organisations to make submissions on this application.

The draft variation was considered for approval by the FSANZ Board having regard to all submissions made during the call for submissions period.

2.5 FSANZ Act assessment requirements

2.5.1 Section 29

2.5.1.1 Consideration of costs and benefits

The Office of Impact Analysis (OIA), formerly OBPR[6], granted FSANZ a standing exemption from the requirement to develop a Regulatory Impact Statement for applications relating to new processing aids and GM foods (OBPR correspondence dated 24 November 2010, reference 12065). This standing exemption was provided as permitting new processing aids and GM foods is deregulatory as their use will be voluntary if the application concerned is approved. This standing exception relates to the introduction of a food to the food supply that has been determined to be safe.

[6] Office of Best Practice Regulation

FSANZ, however, gave consideration to the costs and benefits that may arise from the proposed measure for the purposes of meeting FSANZ Act considerations. The FSANZ Act requires FSANZ to have regard to whether costs that would arise from the proposed measure outweigh the direct and indirect benefits to the community, government or industry that would arise from the proposed measure (paragraph 29(2)(a)).

The purpose of this consideration was to determine if the community, government, and industry as a whole is likely to benefit, on balance, from a move from the status quo, where the status quo is rejecting the application. This analysis considered the costs and benefits of permitting the three GM enzymes for the bioconversion production method of two steviol glycosides, rebaudiosides M and I and the bioconversion method of producing such rebaudiosides.

The consideration of the costs and benefits in this section was not intended to be an exhaustive, quantitative economic analysis of the proposed measures and, in fact, most of the effects that were considered cannot easily be assigned a dollar value. Rather, the assessment sought to highlight the potential positives and negatives of moving away from the status quo by permitting the proposed enzymes for the production of steviol glycosides, rebaudiosides M and I and the bioconversion method of producing such rebaudiosides.

FSANZ's conclusions regarding costs and benefits of the proposed measure are set out below.

Costs and benefits of permitting the use of three GM enzymes for the bioconversion production method of two steviol glycosides, rebaudiosides M and I and the bioconversion method of producing such rebaudiosides

Industry

The proposed new enzymes would be used as processing aids to produce certain food additives i.e. steviol glycosides which are low-calorie sweeteners for a range of foods. The specific steviol glycosides being rebaudiosides M and I. There are other methods of producing rebaudiosides M and I and other steviol glycosides. Industry may adopt this method for producing these rebaudiosides, using the proposed new enzymes as processing aids, if there was a net benefit for them. Benefits may include lower costs and higher efficiency of producing these rebaudiosides using this production method.

Consumers

Industry may pass some of any cost savings to consumers, where it is cheaper to produce these rebaudiosides using the new enzymes as processing aids.

Given the already wide permissions for use of steviol glycosides in foods, it is not currently clear whether or not approval of the draft variation would notably increase availability of lower calorie food products for consumers as claimed by the applicant. If the draft variation is approved, that would, however, be supportive of enabling the range of such food products to continue.

Government

Permitting the new rebaudiosides and the enzymes as processing aids to produce them may result in a small cost to government in terms of additions to the current range of enzymes and rebaudiosides that are monitored for compliance.

Conclusions from cost benefit considerations

FSANZ's assessment conclusion was that the direct and indirect benefits that would arise from permitting the new rebaudiosides and the enzymes as processing aids to produce them would most likely outweigh the associated costs. No further information was received during the consultation process that changed that assessment

2.5.1.2 Other measures

There are no other measures (whether available to FSANZ or not) that would be more cost-effective than a food regulatory measure developed or varied as a result of the application.

2.5.1.3 Any relevant New Zealand standards

The relevant standards apply in both Australia and New Zealand. There are no relevant New Zealand only Standards.

2.5.1.4 Any other relevant matters

Other relevant matters are considered below.

2.5.2 Subsection 18(1)

FSANZ has also considered the three objectives in subsection 18(1) of the FSANZ Act during the assessment.

2.5.2.1 Protection of public health and safety

FSANZ undertook a safety assessment (see SD1) and concluded there were no public health and safety concerns associated with permitting the new rebaudiosides M and I and the use of the three GM enzymes as processing aids to produce them.

2.5.2.2 The provision of adequate information relating to food to enable consumers to make informed choices

The labelling requirements for this application are discussed in section 1.3.1.5 of this report.

2.5.2.3 The prevention of misleading or deceptive conduct

There are no issues identified with this application relevant to this objective.

2.5.3 Subsection 18(2) considerations

FSANZ has also had regard to:

- **the need for standards to be based on risk analysis using the best available scientific evidence**

FSANZ used the best available scientific evidence to conduct the risk analysis. The applicant submitted a dossier of information and scientific literature as part of its application. This dossier, together with other relevant technical and scientific information, was considered by FSANZ in assessing the application. The risk assessment is provided in SD1.

- **the promotion of consistency between domestic and international food standards**

There are relevant international specifications for the production method of steviol glycosides which are relevant to this application, being the JECFA framework.

Section S3—35 of the Code sets out the specification for steviol glycosides produced by enzymatic conversion by using specific methods of production listed in the section. The amendment of the approved draft variation adds the method of production of the applicant's steviol glycosides, rebaudiosides M and I in that list, with the applicant's three enzymes and associated microorganisms explicitly listed. In addition, the amendment to the table to subsection S18—9(3) of the approved draft variation lists those three enzymes as permitted processing aids for that specific purpose.

As such, FSANZ considers that the approved draft variation will promote consistency between domestic and international food standards.

- **the desirability of an efficient and internationally competitive food industry**

Permission for the new rebaudiosides and the enzymes as processing aids to produce them would enable Australian and New Zealand food manufacturers to access and use a product assessed as safe that is available to some overseas competitors. This will improve their capacity to compete in overseas markets. See discussion in section 2.5.1.1 above.

- **the promotion of fair trading in food**

No issues were identified for this application relevant to this objective.

- **any written policy guidelines formulated by the Food Ministers' Meeting**

The Ministerial Policy Guideline *Addition to Food of Substances other than Vitamins and Minerals*[7] includes specific order policy principles for substances added to achieve a solely technological function, such as food additives and processing aids. These specific order policy principles state that permission should be granted where:

- the purpose for adding the substance can be articulated clearly by the manufacturer as achieving a solely technological function (i.e. the 'stated purpose')
- the addition of the substance to food is safe for human consumption
- the amounts added are consistent with achieving the technological function
- the substance is added in a quantity and a form which is consistent with delivering the stated purpose
- no nutrition, health or related claims are to be made in regard to the substance.

FSANZ determined that permitting the applicant's bioconversion methods of producing the steviol glycosides: rebaudiosides I and M, using three new protein-engineered enzymes sourced from genetically modified *Escherichia coli* as processing aids, and the use of those three enzymes for that specific purpose, is consistent with these specific order policy principles for 'Technological Function'. All other relevant requirements of the policy guideline are similarly met.

3 References

Codex 2021a, CXG 36-1989,Class Names and the International Numbering System for Food Additives https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B36-1989%252FCXG_036e.pdf Accessed 13 April 2023

Codex 2021b, CXS 192-1995, General Standard for Food Additives (GSFA), https://www.fao.org/fao-

[7] Food regulation website

who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B192-1995%252FCXS_192e.pdf Accessed 13 April 2023

EC (2012) 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. http://data.europa.eu/eli/reg/2012/231/2023-03-22 Updated version at 22/3/23 Accessed 13 March 2023

EC (2016) COMMISSION REGULATION (EU) 2016/1814 of 13 October 2016 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 steviol glycosides (E 960). Off J Eur Union 59(L278):37-41. Accessed 13 March 2023.

FAO 2021. Compendium of Food Additive Specifications. Joint FAO/WHO Expert Committee on Food Additives (JECFA), 91st Meeting, Monograph 26, February 2021, http://www.fao.org/3/cb4737en/cb4737en.pdf Rome, and specifically (Framework for) Steviol Glycosides https://www.fao.org/3/cb8031en/cb8031en.pdf

FCC (2022). Steviol Glycosides. In: Food Chemicals Codex, 13th edition. Rockville (MD): United States Pharmacopeial Convention.

FSANZ 2017, Application A1132, https://www.foodstandards.gov.au/code/applications/Pages/A1132Definition-of-Steviol-Glycosides.aspx

FSANZ 2018, Application A1157, https://www.foodstandards.gov.au/code/applications/Pages/A1157%E2%80%93EnzymaticproductionofRebaudiosideM.aspx

FSANZ 2019a, Application A1172, https://www.foodstandards.gov.au/code/applications/Pages/A1172EnzymaticproductionofRebaudiosideD.aspx

FSANZ 2019b, Application A1176, https://www.foodstandards.gov.au/code/applications/Pages/A1176.aspx

FSANZ 2020, Application A1183, https://www.foodstandards.gov.au/code/applications/Pages/A1183.aspx

FSANZ 2023, Proposal P1061, https://www.foodstandards.gov.au/code/proposals/Pages/P1061-Code-Maintenance-Proposal-2023.aspx

Health Canada (2023). 9. List of Permitted Sweeteners (List of Permitted Food Additives). S.1.2 Steviol glycosides Accessed 13 March 2023.

PureCircle Stevia Institute 2023, Map Infographic Where in the World is Stevia Approved? Accessed 13

Attachments

A. Approved draft variation to the Australia New Zealand Food Standards Code
B. Explanatory Statement
C. Draft variation to the Australia New Zealand Food Standards Code (call for submissions)

Attachment A – Approved draft variation to the Australia New Zealand Food Standards Code

Food Standards (Application A1268 – Steviol glycosides produced by bioconversion using new enzymes produced by GM *Escherichia coli*) Variation

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The Board of Food Standards Australia New Zealand gives notice of the making of this variation under section 92 of the *Food Standards Australia New Zealand Act 1991*. The variation commences on the date specified in clause 3 of this variation.

Dated [To be completed by the Delegate]

[Insert Delegate's name and position title]
Delegate of the Board of Food Standards Australia New Zealand

Note:

This variation will be published in the Commonwealth of Australia Gazette No. FSC XX on XX Month 20XX. This means that this date is the gazettal date for the purposes of clause 3 of the variation.

1 Name

This instrument is the *Food Standards (Application A1268 – Steviol glycosides produced by bioconversion using new enzymes produced by GM* Escherichia coli*) Variation*.

2 Variation to Standards in the *Australia New Zealand Food Standards Code*

The Schedule varies Standards in the *Australia New Zealand Food Standards Code*.

3 Commencement

The variation commences on the date of gazettal.

Schedule

Schedule 3—Identity and purity

[1] Subparagraph S3—35(2)(d)(ii)

Omit the subparagraph, substitute:

(ii) is sourced from *Pichia pastoris* strain UGT-A;

(e) by enzymatic conversion of purified stevia leaf extract to produce rebaudioside M using all of the following protein engineered enzymes:

(i) UTP-glucose-1-phosphate uridylyltransferase (EC 2.7.7.9) sourced from *Escherichia coli* K-12; and
(ii) UDP-glucosyltransferase sourced from *Escherichia coli* K-12; and
(iii) sucrose synthase (EC 2.4.1.13) sourced from *Escherichia coli* K-12;

(f) by enzymatic conversion of purified stevia leaf extract to produce rebaudioside I using both of the following protein engineered enzymes:

(i) UTP-glucose-1-phosphate uridylyltransferase (EC 2.7.7.9) sourced from *Escherichia coli* K-12; and
(ii) sucrose synthase (EC 2.4.1.13) sourced from *Escherichia coli* K-12.

Schedule 18—Processing aids

[2] Subsection S18—9(3) (table)

Insert the following entry for each enzyme in alphabetical order:

Sucrose synthase, protein engineered variant, (EC 2.4.1.13) sourced from *Escherichia coli* K-12 containing the gene for sucrose synthase from *Glycine max*	For the conversion of purified stevia leaf extract to produce one or more of the following: rebaudioside I and rebaudioside M	GMP
Uridine diphosphate (UDP)-glucosyltransferase, protein engineered variant, sourced from *Escherichia coli* K-12 containing the UDP-glucosyltransferase gene from *Oryza sativa*	For the conversion of purified stevia leaf extract to produce rebaudioside M	GMP
Uridine triphosphate (UTP)-glucose-1-phosphate uridylyltransferase, protein engineered variant, (EC 2.7.7.9) sourced from *Escherichia coli* K-12, containing the gene for UTP-glucose-1-phosphate uridylyltransferase from *Bifidobacterium bifidum*	For the conversion of purified stevia leaf extract to produce one or more of the following: rebaudioside I and rebaudioside M	GMP

Attachment B – EXPLANATORY STATEMENT

EXPLANATORY STATEMENT

Food Standards Australia New Zealand Act 1991

Food Standards (Application A1268 – Steviol glycosides produced by bioconversion using new enzymes produced by GM* Escherichia coli*) Variation

1. Authority

Section 13 of the *Food Standards Australia New Zealand Act 1991* (the FSANZ Act) provides that the functions of Food Standards Australia New Zealand (the Authority) include the development of standards and variations of standards for inclusion in the *Australia New Zealand Food Standards Code* (the Code).

Division 1 of Part 3 of the FSANZ Act specifies that the Authority may accept applications for the development or variation of food regulatory measures, including standards. This Division also stipulates the procedure for considering an application for the development or variation of food regulatory measures.

The Authority accepted Application A1268 which sought to amend the Code to permit the use of three new protein engineered enzymes from genetically modified (GM) *Escherichia coli* strain K-12 as processing aids in the bioconversion method of producing steviol glycosides – rebaudiosides I and M. The Authority considered the application in accordance with Division 1 of Part 3 and has approved a draft variation -the *Food Standards (Application A1268 – Steviol glycosides produced by bioconversion using new enzymes produced by GM* Escherichia coli*) Variation.*

Following consideration by the Food Ministers' Meeting (FMM), section 92 of the FSANZ Act stipulates that the Authority must publish a notice about the draft variation.

2. Variation is a legislative instrument

The approved draft variation is a legislative instrument for the purposes of the *Legislation Act 2003* (see section 94 of the FSANZ Act) and is publicly available on the Federal Register of Legislation (www.legislation.gov.au).

This instrument is not subject to the disallowance or sunsetting provisions of the *Legislation Act 2003.* Subsections 44(1) and 54(1) of that Act provide that a legislative instrument is not disallowable or subject to sunsetting if the enabling legislation for the instrument (in this case, the FSANZ Act): (a) facilitates the establishment or operation of an intergovernmental scheme involving the Commonwealth and one or more States; and (b) authorises the instrument to be made for the purposes of the scheme. Regulation 11 of the *Legislation (Exemptions and other Matters) Regulation 2015* also exempts from sunsetting legislative instruments a primary purpose of which is to give effect to an international obligation of Australia.

The FSANZ Act gives effect to an intergovernmental agreement (the Food Regulation Agreement) and facilitates the establishment or operation of an intergovernmental scheme (national uniform food regulation). That Act also gives effect to Australia's obligations under an international agreement between Australia and New Zealand. For these purposes, the Act establishes the Authority to develop food standards for consideration and endorsement by

the FMM. The FMM is established under the Food Regulation Agreement and the international agreement between Australia and New Zealand, and consists of New Zealand, Commonwealth and State/Territory members. If endorsed by the FMM, the food standards on gazettal and registration are incorporated into and become part of Commonwealth, State and Territory and New Zealand food laws. These standards or instruments are then administered, applied and enforced by these jurisdictions' regulators as part of those food laws.

3. Purpose

The Authority has approved a draft variation amending the Code to include the applicant's bioconversion methods of producing the steviol glycosides: rebaudiosides I and M, using three new protein-engineered enzymes sourced from genetically modified *Escherichia coli* as processing aids, and to permit the use of those three enzymes for that specific use

In particular, the draft variation amends section S3—35 (Specification for steviol glycosides produced by enzymatic conversion) and the table to subsection S18—9(3) (permitted processing aids for various technological purposes) of the Code for the above purpose.

4. Documents incorporated by reference

The approved draft variation does not incorporate any documents by reference.

However, existing provisions of the Code incorporate documents by reference that would prescribe identity and purity specifications for the processing aids and food additives (the steviol glycosides rebaudiosides I and M produced by enzymatic conversion) to be permitted by the approved draft variation. Section 1.1.1—15 of the Code requires substances used as processing aids and food additives to comply with any relevant identity and purity specifications listed in Schedule 3 of the Code. Subsection S3—2(1) of Schedule 3 incorporates by reference primary source specifications listed in the following: Joint FAO/WHO Expert Committee on Food Additives (JECFA) Combined Compendium of Food Additive Specifications (FAO JECFA Monographs 26 (2021)); the United States Pharmacopeial Convention (2022) Food chemicals codex (13th edition); and the Commission Regulation (EU) No 231/2012. These include general specifications for the identity and purity parameters of food additives and enzyme preparations used as processing aids in the production of those additives.

5. Consultation

In accordance with the procedure in Division 1 of Part 3 of the FSANZ Act, the Authority's consideration of application A1268 included one round of public consultation following an assessment and the preparation of a draft variation and associated report. Submissions were called for on 15 June 2023 for a six-week consultation period.

The Office of Impact Analysis[8] granted the Authority a standing exemption from the requirement to develop a Regulatory Impact Statement for applications relating to permitting new processing aids and genetically modified foods (OBPR correspondence dated 24 November 2010, reference 12065). This standing exemption was provided as permitting new processing aids and genetically modified foods is deregulatory as their use will be voluntary if the application concerned is approved. This standing exemption relates to the introduction of a food to the food supply that has been determined to be safe.

6. Statement of compatibility with human rights

[8] Formerly known as the Office of Best Practice Regulation (OBPR)

This instrument is exempt from the requirements for a statement of compatibility with human rights as it is a non-disallowable instrument under section 44 of the *Legislation Act 2003*.

7. Variation

Clause 1 of the variation provides that the name of the variation is the *Food Standards (Application A1268 – Steviol glycosides produced by bioconversion using new enzymes produced by GM* Escherichia coli*) Variation*.

Clause 2 of the variation provides that the Code is amended by the Schedule to the variation.

Clause 3 of the variation provides that the variation will commence on the date of gazettal of the instrument.

7.1 Item [1]

Item [1] of the Schedule to the variation amends section S3—35 of the Code, which sets out specifications for steviol glycosides produced by the enzymatic conversion [bioconversion] method of production.

In particular, this item omits subparagraph S3—35(2)(d)(ii), and substitutes it with the following:

- a new subparagraph S3—35(2)(d)(ii) with a semi-colon at the end of the paragraph, instead of a full stop – this is a consequence of inserting new paragraphs S3—35(2)(e) and (f);

- new paragraphs S3—35(2)(e) and (f), which are inserted in alphabetical order:
  - new paragraph (e) refers to a process for the production of rebaudioside M by enzymatic conversion of purified stevia leaf extract by using these three protein engineered enzymes: UTP-glucose-1-phosphate uridylyltransferase (EC 2.7.7.9) sourced from *Escherichia coli* K-12, UDP-glucosyltransferase sourced from *Escherichia coli* K-12, and sucrose synthase (EC 2.4.1.13) sourced from *Escherichia coli* K-12

  - new paragraph (f) refers to a process for the production of rebaudioside I by enzymatic conversion of purified stevia leaf extract by using these two protein engineered enzymes: UTP-glucose-1-phosphate uridylyltransferase (EC 2.7.7.9) sourced from *Escherichia coli* K-12, and sucrose synthase (EC 2.4.1.13) sourced from *Escherichia coli* K-12.

7.2 Item [2]

Item [2] of the Schedule to the variation amends Schedule 18 of the Code by inserting new entries for three new enzymes into the table to subsection S18—9(3). The table lists substances permitted to be used as processing aids for specific technological purposes.

The following protein-engineered enzymes will each be inserted in alphabetical order into column 1 of the table:

- 'Sucrose synthase, protein engineered variant, (EC 2.4.1.13) sourced from *Escherichia coli* K-12 containing the gene for sucrose synthase from *Glycine max*';

- ‘Uridine diphosphate (UDP)-glucosyltransferase, protein engineered variant, sourced from *Escherichia coli* K-12 containing the UDP-glucosyltransferase gene from *Oryza sativa*’; and
- ‘Uridine triphosphate (UTP)-glucose-1-phosphate uridylyltransferase, protein engineered variant, (EC 2.7.7.9) sourced from *Escherichia coli* K-12, containing the gene for UTP-glucose-1-phosphate uridylyltransferase from *Bifidobacterium* bifidum’.

The permitted technological purpose for each enzyme is prescribed in column 2 of the table for the corresponding enzyme.

For both the sucrose synthase and UTP-glucose-1-phosphate uridylyltransferase enzymes, the technological purpose is ‘for the conversion of purified stevia leaf extract to produce one or more of the following: rebaudioside M and rebaudioside I’.

For the UDP-glucosyltransferase enzyme, the technological purpose is ‘for the conversion of purified stevia leaf extract to produce rebaudioside M’.

The maximum permitted level (MPL) at which each enzyme may be present in food is prescribed in column 3 of the table for the corresponding enzyme. For each enzyme inserted into the table in this variation, the MPL specified is GMP i.e. the MPL must be consistent with Good Manufacturing Practice (as defined by subsection 1.1.2—2(3) of the Code).

The cumulative effect of the amendments in items [1] and [2] above would be to permit the applicant’s bioconversion methods of producing the steviol glycosides: rebaudiosides I and M, using three new protein-engineered enzymes sourced from genetically modified *Escherichia coli* as processing aids, and the use of those three enzymes for that specific purpose, in accordance with the Code.

Attachment C – Draft variation to the Australia New Zealand Food Standards Code (call for submissions)

FOOD STANDARDS Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

Food Standards (Application A1268 – Steviol glycosides produced by bioconversion using new enzymes produced by GM *Escherichia coli*) Variation

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The Board of Food Standards Australia New Zealand gives notice of the making of this variation under section 92 of the *Food Standards Australia New Zealand Act 1991*. The variation commences on the date specified in clause 3 of this variation.

Dated [To be completed by the Delegate]

[Insert Delegate's name and position title]
Delegate of the Board of Food Standards Australia New Zealand

Note:

This variation will be published in the Commonwealth of Australia Gazette No. FSC XX on XX Month 20XX. This means that this date is the gazettal date for the purposes of clause 3 of the variation.

1 Name

This instrument is the *Food Standards (Application A1268 – Steviol glycosides produced by bioconversion using new enzymes produced by GM* Escherichia coli*) Variation*.

2 Variation to Standards in the *Australia New Zealand Food Standards Code*

The Schedule varies Standards in the *Australia New Zealand Food Standards Code*.

3 Commencement

The variation commences on the date of gazettal.

Schedule

Schedule 3—Identity and purity

[1] Paragraphs S3—35(1) (definition of *prescribed rebaudiosides*)

Repeal the definition, substitute:

prescribed rebaudiosides are:

(a) rebaudioside AM; and

(b) rebaudioside D; and

(c) rebaudioside I; and

(d) rebaudioside M.

[2] Subparagraph S3—35(2)(c)

Repeal this paragraph, substitute:

(c) by enzymatic conversion of purified stevia leaf extract to produce one or more prescribed rebaudiosides using a combination of enzymes that contains:

(i) a sucrose synthase (EC 2.4.1.13) sourced from *Escherichia coli*; and

(ii) a UDP-glucosyltransferase from *Oryza sativa* sourced from *Escherichia coli*; and

(iii) a UDP-glucosyltransferase from *Solanum lycopersicum* sourced from *Escherichia coli*; and

(iv) a UDP-glucosyltransferase from *Stevia rebaudiana* sourced from *Escherichia coli*; and

(v) a UTP-glucose-1-phosphate uridylyltransferase (EC 2.7.7.9) sourced from *Escherichia coli*.

Schedule 18—Processing aids

[3] Subsection S18—9(3) (table)

Insert each of the following entries in alphabetical order:

Sucrose synthase, protein engineered variant, (EC 2.4.1.13) sourced from *Escherichia coli* K-12 containing the gene for sucrose synthase from *Glycine max*	For the conversion of purified stevia leaf extract to produce one or more of the following: rebaudioside I and rebaudioside M	GMP
Uridine diphosphate (UDP) glucosyltransferase, protein engineered variant, sourced from *Escherichia coli* K-12 containing the UDP glucosyltransferase gene from *Oryza sativa*	For the conversion of purified stevia leaf extract to produce one or more of the following: rebaudioside I and rebaudioside M	GMP

Uridine triphosphate (UTP)-glucose-1-phosphate uridylyltransferase, protein engineered variant, (EC 2.7.7.9) sourced from *Escherichia coli* K-12, expressing the gene for UTP-glucose-1-phosphate uridylyltransferase from *Bifidobacterium bifidum*	For the conversion of purified stevia leaf extract to produce one or more of the following: rebaudioside I and rebaudioside M	GMP