Assessment for the Application for a change in the Steviol Glycoside Specification in Great Britain to Include a New Manufacturing Method for Steviol Glycosides Including Rebaudioside M.

Area of research interest: Novel and non-traditional foods, additives and processes
Planned completion: 17 October 2022
Project status: Completed
Project code: RP 1194
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Conducted by: Food Standards Agency and Food Standards Scotland

1. Executive Summary

The FSA/FSS have undertaken an assessment of application RP 1194 for a change in the steviol glycoside specification in the United Kingdom to include a new manufacturing method for steviol glycosides including Rebaudioside M, from Sweegen, Inc.

A food additive application has been received by Great Britain (GB) where the European Food Safety Authority (EFSA), prior to the end of the transition period, evaluated an application for the product. FSA/FSS has reviewed the EFSA opinion (EFSA Journal 2019;17(10):5867) for this food additive and confirmed that it is adequate and relevant for GB risk analysis. Therefore, the EFSA opinion was used to form the basis of the GB opinion.

The FSA/FSS risk assessors concluded that the EFSA opinion is adequate and relevant for the risk analysis and therefore accepts the EFSA conclusion that there is no safety concern for Rebaudioside M produced via enzymatic bioconversion of purified stevia leaf extract using UDP-glucosyltransferase and sucrose synthase enzymes produced by the genetically modified yeasts K. phaffii UGT-a and K. phaffii UGT-b.

2. Background and purpose of review

EFSA Journal 2019;17(10):5867

Question number: EFSA-Q-2018-00242

In accordance with Retained EU Regulation 1331/2008, establishing a common authorisation procedure for food additives, food enzymes and food flavourings, the application RP 1194 for a proposed amendment of the specifications of the food additive steviol glycosides (E 960), from Sweegen, Inc. has been submitted for authorisation in each nation of GB.

Specifically, the application requests an amendment of the present specifications of Steviol glycosides (E 960) to include a new production process for rebaudioside M produced via enzymatic bioconversion of purified stevia leaf extract (?95% steviol glycosides) using the enzymes Uridine 5'-diphospho(UDP)-glucosyltransferase and sucrose synthase.

Whilst it was a Member State of the EU, the UK accepted the assessments of the European Food Safety Authority (EFSA) in respect of authorisations for regulated food and feed products. Since the end of the transition period, FSA/FSS has adopted equivalent technical guidance and quality assurance processes to be able to undertake GB risk assessments for regulated product applications. Where EFSA, prior to the end of the transition period, evaluated an application for a product for which an application is now made to GB, FSA/FSS has decided to make use of the EFSA risk assessment, where this is appropriate, in forming its own opinion. Therefore, FSA/FSS risk assessors have reviewed the EFSA opinions for the application below in the context of intended GB use and have concluded that the intended uses are safe.

In reviewing the output of the EFSA risk assessment the reviewers have verified that the standard approach as outlined in the relevant guidance (footnote 1) (footnote 2), (footnote 3) has been followed and the arguments made are consistent with the data summarised. Consideration has been given to the processes undertaken by EFSA to ensure the outcome is robust and whether there are any aspects that would require further review such as specific issues for the countries of the GB. The result of the assessment is that the EFSA scientific opinion is adequate for GB risk analysis.

3. Details of the EFSA Assessment

3.1 Methodology applied in the EFSA opinion

The EFSA Opinion was formulated based on the 'Guidance for submission for food additive evaluation' (EFSA ANS Panel, 2012).

'Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use' (EFSA GMO Panel, 2011) and 'Guidance on the characterisation of microorganisms used as feed additives or as production organisms' (EFSA FEEDAP Panel, 2018) have been followed by the FAF Panel for evaluating the proposed change in manufacturing process and changes in the specifications.

3.2 Source/organism

The proposed manufacturing method is the production of rebaudioside M via enzyme-catalysed bioconversion of purified stevia leaf extract.

The applicant states that Rebaudioside M (?95%) is manufactured through a multistep enzymatic process using UDP-glucosyltransferase and sucrose synthase enzymes produced by the genetically modified yeast strains K. phaffii UGT-a and K phaffii UGT-b, respectively.

3.3 Genetic modification step

Information on the characterisation of the producing organism (including characteristics of the parental and recipient microorganisms, characteristics of inserted sequences and description of the genetic modification process) have been provided in the Opinion. However, the Applicant has demonstrated the absence of the production microorganisms in the final rebaudioside M product in three independent batches analysed in triplicate. Additionally, the manufacturing process outlined all the steps to inactivate and remove the enzyme system. The final rebaudioside M product was tested for residual protein using the bicinchoninic acid (BCA) assay to ensure that the processing enzymes were effectively removed, and no residual protein was detected (limit of

detection = 5 ppm).

Since the GMO organism is not present in the final product, a risk assessment on the GMO enzymes is not required.

3.4 Specification

The Applicant has proposed two changes to the specifications for E960 steviol glycoside. These are described below:

1. An expansion of the definition to include enzymatic bioconversion of purified stevia leaf extract as a permitted manufacturing method for rebaudioside M. The following text is proposed to be added to the current definition for E960: 'In order to produce a higher yield of rebaudioside M, purified steviol glycoside leaf extracts may be subject to enzymatic bioconversion, utilising-glucosyltransferase and sucrose synthase enzymes derived from strains of K. phaffii that facilitate the transfer of glucose to steviol glycosides via glycosidic bonds'.

2. For rebaudioside M produced by enzymatic bioconversion of purified stevia leaf extract, an additional purity parameter is proposed to be added to ensure that no residual DNA arising from the use of enzymatic processing aids derived from K. phaffii (i.e. UDP-glucosyltransferase and sucrose synthase enzymes derived from strains of K. phaffii is present in the final product. The following text is proposed to be added to the end of the current list of purity parameters: 'Residual DNA, Not more than 10 ng DNA/g product [only applicable to rebaudioside M produced by enzymatic bioconversion of purified stevia leaf extract]'.
Information on the proposed specifications are included in Appendix A of the EFSA Opinion.

With regards to the proposed definition of 'purified steviol glycoside leaf extracts may be subject to enzymatic bioconversion, utilising UDP-glucosyltransferase and sucrose synthase enzymes derived from strains of K. phaffii that facilitate the transfer of glucose to steviol glycosides via glycosidic bonds', the Panel considered that information on all the sources (e.g. transfer of glucose from sucrose and UDP-glucose) for this manufacturing process as well as the specific strains of K. phaffii (K. phaffii UGT-a and K. phaffii UGT-b) should be mentioned in the definition.

The Panel noted that the limit for residual DNA would be valid only for rebaudioside M produced by enzymatic bioconversion of purified stevia leaf extract and it is not applicable for steviol glycosides extracted from the leaves of Stevia rebaudiana. The Panel considered that this reconfirmed the need for separate specifications

Additional information was provided on possible microbiological contaminants indicating that these were either at levels below the current specifications or absent.

With regards to the information received on chemical contaminants, the Panel considered that that maximum limits for cadmium and mercury should be added to the proposed specification and lower levels of the maximum limits for lead and arsenic could be considered in the proposed specification.

The presence of impurities such as ethanol, kaurenoic acid, presence of pesticide residues residual protein as well as particle size information have also been considered in the Opinion.

3.5 Exposure assessment

Not relevant.

3.6 Toxicological data

The Applicant submitted to EFSA, scientific publications which were considered relevant to the safety of steviol glycoside. Biological and toxicological studies not performed with rebaudioside M produced via enzymatic bioconversion of purified stevia leaf extract or rebaudioside M from stevia extract were not considered by the Panel as they were not considered relevant to the substance under evaluation.

4. EFSA assessment and conclusions

The EFSA FAF Panel concluded that there is no safety concern for Rebaudioside M produced via enzymatic bioconversion of purified stevia leaf extract using UDP-glucosyltransferase and sucrose synthase enzymes produced by the genetically modified yeasts K. phaffii UGT-a and K. Phaffii UGT-b, to be used as a food additive. However, the Panel recommended that the European Commission consider establishing separate specifications for Rebaudioside M produced via enzymatic bioconversion of purified stevia leaf extract in Commission Regulation (EU) No 231/2012.

5. Caveats and uncertainties

Information on the manufacturing process is not included in the EFSA Opinion as it is proprietary to the Applicant. The applicant has supplied this information to GB as part of their application and this did not raise any concerns.

Although the studies used to support the bacterial conversion of Rebaudioside M to the common metabolite could have been improved, it was noted that this provided a similar level of evidence to other previously approved steviol glycosides and therefore supported the case for the safety of the product seeking authorisation.

6. FSA/FSS Conclusion on applicability and reliability of the EFSA opinion for GB risk analysis

FSA and FSS have access to the full dossier of information from the applicant but have used the EFSA opinion as the basis of their assessment. The FSA and FSS assessment of the EFSA opinion has confirmed the application has been assessed in line with the applicable guidance and is partially based on considerations of detailed proprietary information available to the EFSA Panel, whilst this is only briefly summarised in the EFSA opinion, this description is consistent with the conclusions. The EFSA conclusions are appropriate and consistent within the identified caveats and uncertainties and would be applicable to GB.

7. Outcome of assessment

FSA/FSS risk assessors have reviewed the EFSA opinion for a change in the steviol glycoside specification in the GB to include a new manufacturing method for steviol glycosides including Rebaudioside M, from Sweegen, Inc. and consider it adequate and relevant for GB risk analysis. Therefore, the EFSA opinion was used to form the basis of the GB opinion.

FSA/FSS had access to the full dossier of information for the application supplied by the applicant but have used the EFSA opinion as the basis of their assessment. FSA/FSS agree with the safety conclusions outlined in the EFSA opinion.

FSA/FSS conclude that Rebaudioside M produced via enzymatic bioconversion of purified stevia leaf extract using UDP-glucosyltransferase and sucrose synthase enzymes produced by the genetically modified yeasts K. phaffii UGT-a and K. Phaffii UGT-b used as a food additive does not raise any safety concerns.

FSA/FSS also agreed with EFSA FAF Panel's recommendation for risk managers that separate specifications for Rebaudioside M produced via enzymatic bioconversion of purified stevia leaf extract, be considered in the authorisation decision, to distinguish this from similar products produced using different production methods.

8. References

- EFSA (European Food Safety Authority), (2012) Guidance for submission for food additive evaluations. EFSA Journal 2012;10(7):2760, DOI: https://doi.org/10.2903/j.efsa.2012.2760
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- EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources), 2012. Guidance for submission for food additive evaluations. EFSA Journal 2012;10(7):2760
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2018. Guidance on the characterisation of microorganisms used as feed additives or as production organisms. EFSA Journal2018;16(3):5206
- EFSA GMO Panel (EFSA Panel on Genetically Modified Organism), 2011. Guidance for the risk assessment of genetically modified microorganism and their products intended for food and feed use. EFSA Journal 2011;9(6):2103

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2. European Food Safety Authority (EFSA), (2009) Data requirements for the evaluation of food additive applications, EFSA Journal (2009) 1188, 1-7 .

3. EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources), 2012. Guidance for submission for food additive evaluations. EFSA Journal 2012;10(7):2760