Adopted: 13 March 2024

DOI: 10.2903/j.efsa.2024.8702

SCIENTIFIC OPINION

efsa JOURNAL

Safety evaluation of the food enzyme preparation D-psicose 3-epimerase from the non-genetically modified *Microbacterium foliorum* strain SYG27B

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) | Claude Lambré | José Manuel Barat Baviera | Claudia Bolognesi | Pier Sandro Cocconcelli | Riccardo Crebelli | David Michael Gott | Konrad Grob | Evgenia Lampi | Marcel Mengelers | Alicja Mortensen | Gilles Rivière | Inger-Lise Steffensen | Christina Tlustos | Henk Van Loveren | Laurence Vernis | Holger Zorn | Magdalena Andryszkiewicz | Laura Sanmartin Cabo | Ana Criado | Cristina Fernàndez-Fraguas | Yi Liu | Silvia Peluso | Rita Ferreira de Sousa | Andrew Chesson

Correspondence: fip@efsa.europa.eu

Abstract

This assessment addresses a food enzyme preparation consisting of the immobilised non-viable cells of the non-genetically modified bacterium identified by the applicant (Samyang Corporation) as *Microbacterium foliorum* strain SYG27B. This strain produces the enzyme D-psicose 3-epimerase (EC 5.1.3.30). The food enzyme preparation is used for the isomerisation of fructose to produce the speciality carbohydrate D-allulose (synonym D-psicose). Since the hazard identification and characterisation could not be made and the identity of the production organism could not be established, the Panel was unable to complete the assessment of this food enzyme preparation containing D-psicose 3-epimerase.

KEYWORDS

D-allulose 3-epimerase, D-psicose 3-epimerase, EC 5.1.3.30, food enzyme, *Microbacterium foliorum*

CONTENTS

1 | INTRODUCTION

Article 3 of the Regulation (EC) No 1332/2008[1] provides definition for 'food enzyme' and 'food enzyme preparation'.

'Food enzyme' means a product obtained from plants, animals or microorganisms or products thereof including a product obtained by a fermentation process using microorganisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.

'Food enzyme preparation' means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

Before January 2009, food enzymes other than those used as food additives were not regulated or were regulated as processing aids under the legislation of the Member States. On 20 January 2009, Regulation (EC) No 1332/2008 on food enzymes came into force. This Regulation applies to enzymes that are added to food to perform a technological function in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food, including enzymes used as processing aids. Regulation (EC) No 1331/2008[2] established the European Union (EU) procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

- it does not pose a safety concern to the health of the consumer at the level of use proposed;
- there is a reasonable technological need;
- its use does not mislead the consumer.

All food enzymes currently on the EU market and intended to remain on that market, as well as all new food enzymes, shall be subjected to a safety evaluation by the European Food Safety Authority (EFSA) and approval via an EU Community list.

The 'Guidance on submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) lays down the administrative, technical and toxicological data required.

1.1 | Background and Terms of Reference as provided by the requestor

1.1.1 | Background as provided by the European Commission

Only food enzymes included in the Union list may be placed on the market as such and used I foods, in accordance with the specification and condition of use provided for in Article 7 (2) of Regulation (EC) No 1332/2008 on food enzymes.[3]

On 6 June 2021, a new application has been introduced by the applicant "Samyang Corporation" for the authorization of the food enzyme D-psicose 3-epimerase from a non-genetically modified strain of *Microbacterium foliorum* (strain SYG27B).

1.1.2 | Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessment and the assessment of possible confidentiality requests of the following food enzyme: D-psicose 3-epimerase from a non-genetically modified strain of *Microbacterium foliorum* (strain SYG27B), in accordance with Regulation (EC) No 1331/2008 establishing a common authorization procedure for food additives, food enzymes and food flavourings.

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme D-psicose 3-epimerase from a non-genetically modified *Microbacterium foliorum* (strain SYG27B).

[1]Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on Food Enzymes and Amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L 354, 31.12.2008, pp. 7–15.

[2]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. OJ L 354, 31.12.2008, pp. 1–6.

[3]Commission Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 64, 11.03.2011, pp. 15–24.

2.2 | Methodologies

The assessment was conducted in line with the principles described in the EFSA 'Guidance on transparency in the scientific aspects of risk assessment' (EFSA, 2009b) and following the relevant guidance documents of the EFSA Scientific Committee.

The 'Scientific Guidance for the submission of dossiers on Food Enzymes' (EFSA CEP Panel, 2021) and the guidance on 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023) have been followed for the evaluation of the application.

2.3 | Public consultation

According to Article 32c(2) of Regulation (EC) No 178/2002[4] and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations, EFSA carried out a public consultation on the non-confidential version of the technical dossier from 15 January to 5 February 2024.[5] No comments were received.

3 | ASSESSMENT

IUBMB nomenclature	D-psicose 3-epimerase
Systematic name	D-psicose 3-epimerase
Synonyms	D-allulose 3-epimerase; fructose epimerase
IUBMB No	EC 5.1.3.30
CAS No	1219591-85-1
EINECS No	807-816-3

D-Psicose 3-epimerases catalyse the epimerisation of D-fructose at the C3 position to produce D-allulose (also known as D-psicose). The enzyme under assessment is retained in the non-viable *Microbacterium foliorum* SYG27B cells, which are used to produce D-allulose.

3.1 | Source of the food enzyme

The D-psicose 3-epimerase is produced with the non-genetically modified bacterium identified by the applicant as *Microbacterium foliorum* strain SYG27B, which is deposited at the Korean Culture Center of Microorganisms (KCCM, South Korea) with the deposit number [redacted].[6]

M. foliorum SYG27B was isolated from ginseng.[7] The identification provided by the applicant was based on 16S rRNA gene analysis, which the Panel considered insufficient to conclusively establish the identity of the production strain.[8] Despite being requested, the applicant did not provide the identification based on whole genome sequencing (WGS) data, but rather re-sent the 16S rRNA gene analysis.[9]

The absence of genes encoding for pathogenicity/virulence was demonstrated by analysing the WGS. The WGS of the production strain was also interrogated for the presence of antimicrobial resistance genes with thresholds of [redacted] identity and [redacted] coverage. No genes of concern were identified.[10,11]

3.2 | Production of the food enzyme preparation

The food enzyme preparation is manufactured according to the Food Hygiene Regulation (EC) No 852/2004,[12] with food safety procedures based on Hazard Analysis and Critical Control Points and in accordance with current good manufacturing practice.

The production strain is grown as a pure culture using a typical industrial medium in a submerged, fed-batch fermentation system with conventional process controls in place. After completion of the fermentation, cells are washed with water

[4]6 Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, pp. 1–24.

[5]Accessible at: https://connect.efsa.europa.eu/RM/s/publicconsultation2/a0lTk0000004aT7/pc0777.

[6]Technical dossier/Risk Assessment/Source of the food enzyme/Annex C p. 20.

[7]Technical dossier/Detailed summary.

[8]Technical dossier/Risk Assessment/Source of the food enzyme/Annex C.

[9]Additional data September 2023/ Source of the food enzyme/Annex D-1. (Additional data provided, but it included the data delivered in the original submission).

[10]Technical dossier/Risk Assessment/Source of the food enzyme/Annex G.

[11]Additional data September 2023/ Source of the food enzyme/Annex O.

[12]Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of food additives. OJ L 226, 25.6.2004, pp. 3–21.

to remove the fermentation medium, killed by treating with the emulsifier [redacted] at [redacted] for [redacted] and harvested by centrifugation. The cells containing the enzyme are then mixed with an alginic acid solution. The mixture is [redacted], extruded and [redacted] a [redacted] solution to form beads.[13] The beads are then dried.[14] The applicant provided information on the identity of the substances used to control the fermentation and in the subsequent downstream processing of the food enzyme preparation.

The Panel considered that sufficient information has been provided on the manufacturing process and the quality assurance system implemented by the applicant to exclude issues of concern.

3.3 | Characteristics of the food enzyme

3.3.1 | Properties of the food enzyme

The D-psicose 3-epimerase is a single polypeptide chain of [redacted] amino acids.[15] The molecular mass of the mature protein, calculated from the amino acid sequence, is around [redacted].

The in-house determination of D-psicose-3-epimerase activity is based on the conversion of D-fructose to D-allulose (reaction conditions: [redacted], [redacted], [redacted]) that is quantified by high-performance liquid chromatography (HPLC) with [redacted]. The enzyme activity is expressed in Units/g (U/g). One unit of psicose 3-epimerase activity is defined as the amount of dry cells (g) that catalyse the production of 1 μmol of D-allulose per minute.[16]

The food enzyme has a temperature optimum at [redacted] ([redacted]) and a pH optimum at [redacted] ([redacted]), the highest pH tested. Thermostability was tested after a pre-incubation of the food enzyme at [redacted] for different time periods ([redacted]). The enzyme activity decreased by [redacted] after [redacted] of pre-incubation.[17]

3.3.2 | Chemical parameters

Data on the chemical parameters of the food enzyme preparation were provided for five batches used for commercialisation (Table 1). The mean total organic solids (TOS) of the five food enzyme batches was 19.5% and the mean enzyme activity/TOS ratio was 1.6 UNIT/mg TOS.

TABLE 1 Composition of the food enzyme preparation.[18]

Parameters	Unit	Batches				
		1	2	3	4	5
D-psicose 3-epimerase activity	U/g *M. foliorum* cww[a]	310.25	307.22	318.49	313.99	310.96
Protein	%	14.2	14.2	14.2	14.2	14.3
Ash	%	1.3	1.3	1.2	1.2	1.2
Water	%	79.2	79.3	79.2	79.1	79.4
Total organic solids (TOS)[b]	%	19.5	19.4	19.6	19.7	19.4
Activity/TOS ratio	UNIT/mg TOS	1.6	1.6	1.6	1.6	1.6

[a]U: Unit of psicose 3-epimerase activity (cww = cell wet weight) (see Section 3.3.1).

[b]TOS calculated as 100% – % water – % ash.

3.3.3 | Purity

The lead content in the five commercial batches was below 5 mg/kg, which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). In addition, arsenic, cadmium and mercury contents were below the limits of quantification (LoQs) of the employed methods.[19,20]

[13]Technical dossier/Risk assessment/Manufacturing process of the food enzyme/Technical dossier.

[14]Technical dossier/Manufacturing process of the food enzyme/Technical dossier/p6.

[15]Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/Annex A.

[16]Technical dossier/Risk assessment/Methods of analysis/Technical dossier.

[17]Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/ Chemical composition.

[18]Technical dossier/Risk assessment/ Chemical composition, properties and purity of the food enzyme/Annex B & Chemical composition p. 3.

[19]LoQs: Pb = 0.05 mg/kg; As = 0.05 mg/kg; Cd = 0.04 mg/kg; Hg = 50 μg/kg.

[20]Technical dossier/Risk assesment/ Chemical composition, properties and purity of the food enzyme/Annex B.

The food enzyme preparation complies with the microbiological criteria for total coliforms, *Escherichia coli* and *Salmonella*, as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). No antimicrobial activity was detected in any of the tested batches.[21]

The Panel considered that the information provided on the purity of the food enzyme was sufficient.

3.3.4 | Viable cells of the production strain

The absence of viable cells of the production strain in the food enzyme preparation was assessed in three independent batches analysed in triplicate. [redacted] of beads of alginate-immobilised cells were crushed and resuspended in [redacted] water. From this, an unknown volume of this suspension was diluted and plated onto agar plates, which were incubated for [redacted] at [redacted]. No colonies were produced. Due to uncertainty on the amount of the enzyme tested, the absence of viable cells of the production strain in the food enzyme preparation could not be demonstrated.[22]

3.4 | Toxicological data

A battery of toxicological tests has been provided, including a bacterial reverse mutation test (Ames test), an in vitro mammalian chromosomal aberration, an in vivo mouse micronucleus tests and a repeated dose 90-day oral toxicity study in rats. The applicant was requested to provide the data which would allow a comparison to be made of the material used in these studies with the commercial batches shown in Table 1, and thus a judgement to be made on the suitability of the test item. These data were not provided and consequently the toxicological studies were not further considered.

3.4.1 | Allergenicity

The allergenicity assessment considered only the D-psicose 3-epimerase and not carriers or other excipients that may be used in the final formulation.

The potential allergenicity of the enzyme D-psicose 3-epimerase produced with *Microbacterium foliorum* strain SYG27B was assessed by comparing its amino acid sequence with those of known allergens according to the 'Scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms' (EFSA GMO Panel, 2010). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, no matches were found.[23]

No information is available on oral and respiratory sensitisation or elicitation reactions of this enzyme. In addition, no allergic reactions to ingestion or respiratory exposure to epimerases have been reported in the literature.

[redacted], a known source of allergens, is present in the media fed to the microorganisms.[24] However, during the fermentation process, this product will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the microbial/fungal biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that no potentially allergenic residues from this source are present in the food enzyme.

The Panel considered that the risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded, but the likelihood is low.

3.5 | Dietary exposure

3.5.1 | Intended use of the food enzyme

The non-viable cells containing the food enzyme are intended to be used in immobilised form to produce D-allulose at a recommended use level up to 27 mg TOS/kg D-fructose.[25]

As the food enzyme is intended to be used only in immobilised form, the transfer of food enzyme-TOS into the final product, i.e. highly purified D-allulose, is expected to be limited. Following the epimerisation step, the reaction product (non-purified D-allulose) is subjected to a series of purification steps (including ion exchange chromatography) to obtain the D-allulose syrup, which is then crystallised to obtain the crystalline D-allulose. It can be expected that residual amounts

[21]Additional data September 2023/Chemical composition, properties and purity of the food enzyme/Annex R.

[22]Additional data September 2023/Source of the food enzyme/Annex P.

[23]Technical dossier/Risk Assessment/Allergenicity/Annex A.

[24]Technical dossier/Risk Assessment/ Manufacturing process of the food enzyme/Technical dossier.

[25]Additional information September 2023/Answer 17.

of TOS in the crystalline D-allulose is negligible. However, no analytical data were provided to establish the extent of removal in the allulose syrups.[26]

The applicant provided polymerase chain reaction (PCR) analysis of DNA from the production strain in three types of D-allulose products ([redacted]). No DNA was detected.[27] However, the Panel considered that PCR analysis is not adequate to demonstrate the absence of the food enzyme-TOS in the final food product.

3.5.2 | Dietary exposure estimation

Since the identity of the production organism could not be established and the hazard identification and characterisation could not be completed, the Panel decided not to proceed to calculate the dietary exposure.

4 | CONCLUSIONS

Since the hazard identification and characterisation could not be made and the identity of the production organism could not be established, the Panel was unable to complete the assessment of the food enzyme preparation containing D-psicose 3-epimerase produced with the non-genetically modified bacterium identified by the applicant as *Microbacterium foliorum* strain SYG27B.

5 | DOCUMENTATION AS PROVIDED TO EFSA

Application for the authorisation of D-psicose 3-epimerase naturally present in non-GMM *Microbacterium foliorum* SYG27B. January 2023. Submitted by Samyang Corporation.

Additional information. September 2023. Submitted by Samyang Corporation.

ABBREVIATIONS

CAS	Chemical Abstracts Service
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EC	European Commission
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural OrganisationOrganization of the United Nations
GMM	genetically modified microorganism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
PCR	polymerase chain reaction
TOS	total organic solids
WGS	whole genome sequencing
WHO	World Health Organization

CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

REQUESTOR

European Commission

QUESTION NUMBER

EFSA-Q-2021-00653

[26] Additional information September 2023.

[27] Technical dossier/Risk Assessment/Source of the food enzyme/Annex F.

PANEL MEMBERS

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FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization). (2006). *General specifications and considerations for enzyme preparations used in food processing in compendium of food additive specifications*. 67th meeting FAO JECFA Monographs, 3, 63–67. http://www.fao.org/3/a-a0675e.pdf

How to cite this article: EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré, C., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Andryszkiewicz, M., Cabo, L. S., … Chesson, A. (2024). Safety evaluation of the food enzyme preparation D-psicose 3-epimerase from the non-genetically modified *Microbacterium foliorum* strain SYG27B. *EFSA Journal*, *22*(4), e8702. https://doi.org/10.2903/j.efsa.2024.8702