Accepted: 12 March 2024

DOI: 10.2903/j.efsa.2024.8701

efsa JOURNAL

SCIENTIFIC OPINION

Safety evaluation of an extension of use of the food enzyme endo-polygalacturonase from the genetically modified *Aspergillus oryzae* strain AR-183

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Abstract

The food enzyme endo-polygalacturonase ((1 → 4)-α-D-galacturonan glycanohydrolase EC 3.2.1.15) is produced with the genetically modified *Aspergillus oryzae* strain AR-183 by AB ENZYMES GmbH. A safety evaluation of this food enzyme was made previously, in which EFSA concluded that this food enzyme did not give rise to safety concerns when used in five food manufacturing processes. Subsequently, the applicant requested to extend its use to two additional processes. In this assessment, EFSA updated the safety evaluation of this food enzyme for use in a total of seven food manufacturing processes. As the food enzyme-total organic solids (TOS) is removed from the final foods in three food manufacturing processes, the dietary exposure to the food enzyme-TOS was estimated only for the remaining four processes. Dietary exposure was up to 0.087 mg TOS/kg body weight (bw) per day in European populations. When combined with the NOAEL reported in the previous opinion (1000 mg TOS/kg bw per day, the highest dose tested), the Panel derived a margin of exposure of at least 11,494. Based on the data provided for the previous evaluation and the revised margin of exposure, the Panel concluded that this food enzyme does not give rise to safety concerns under the revised intended conditions of use.

KEYWORDS

Aspergillus oryzae, EC 3.2.1.15, EFSA-Q-2021-00066, EFSA-Q-2023-00524, endo-polygalacturonase, food enzyme, genetically modified microorganism

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 European Union 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.

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 in foods, in accordance with the specifications and conditions of use provided for in Article 7 (2) of Regulation (EC) No 1332/2008[1] on food enzymes.

Polygalacturonase from a genetically modified strain of *Aspergillus oryzae* (strain AR-183) is a food enzyme to be considered for inclusion in the Union list and thus subject to a risk assessment by the European Food Safety Authority (EFSA).

On 24 March 2023, a new application has been introduced by the applicant 'AB Enzymes Gmbh' for the extension of the conditions of use for the above food enzyme in several food processes.

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 an extension of the condition of use for the following food enzyme: Polygalacturonase from a genetically modified strain of *Aspergillus oryzae* (strain AR-183), in accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings.[3]

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for the authorisation of the extension of use of food enzyme polygalacturonase from a genetically modified *Aspergillus oryzae* strain AR-183.

[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]OJ L 354, 31.12.2008, p. 1.

Additional information was requested from the applicant during the assessment process on 30 October 2023 and received on 20 November 2023. Spontaneous data submission was received from the applicant on 19 February 2024 (see 'Documentation provided to EFSA').

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, 2009) and following the relevant existing guidance documents of EFSA Scientific Committee.

The 'Scientific Guidance for the submission of dossiers on Food Enzymes' (EFSA CEP Panel, 2021) and the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023a) have been followed to evaluate this 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 November to 06 December 2023.[5] No comments were received.

3 | ASSESSMENT

IUBMB nomenclature	Endo-polygalacturonase
Systematic name	(1→4)-α-D-galacturonan glycanohydrolase
Synonyms	Pectinase, pectin hydrolase, endo-D-galacturonase
IUBMB no	EC 3.2.1.15
CAS no	9032-75-1
EINECS no	232-885-6

Endo-polygalacturonases catalyse the random hydrolysis of α–(1–4) glycosidic bonds between galacturonic acid residues in polygalacturonans, resulting in their progressive depolymerisation.

All aspects concerning the safety of this food enzyme were evaluated in February 2023, when used in five food manufacturing processes (EFSA CEP Panel, 2023b). Following an application for two additional food manufacturing processes, EFSA revises the exposure assessment and updates the safety evaluation of this food enzyme when used in seven food manufacturing processes.

3.1 | Dietary exposure

The current dietary exposure supersedes section 3.5 of the previous evaluation (EFSA CEP Panel, 2023b).

3.1.1 | Revised intended use of the food enzyme

The food enzyme is intended to be used in seven food manufacturing processes at the use levels summarised in Table 1.

[4]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, p. 1–24.

[5]https://connect.efsa.europa.eu/RM/s/publicconsultation2/a0lTk0000000gqT/pc0714

TABLE 1 Updated intended uses and use levels of the food enzyme.[6]

Food manufacturing process[a]	Raw material (RM)	Maximum recommended use level (mg TOS/kg RM)	
		Current evaluation[b]	Previous evaluation[b,c]
Processing of fruits and vegetables			
• Production of juices	Fruit and vegetables	**2**	**2**
• Production of fruit and vegetable products other than juices	Fruit and vegetables	**1**	**1**
• Production of wine and wine vinegar	Grapes	**2**	**2**
• Production of distilled alcoholic beverages[d]	Fruit	2	
• Production of alcoholic beverages other than from grape	Fruit	**2**	
Processing of plant- and fungal-derived products			
• Production of green coffee beans by demucilation	Coffee cherry	0.5	0.5
• Production of plant extracts as flavouring preparations	Fruit and vegetables	1.5	1.5

[a]The name has been harmonised by EFSA according to the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023a).

[b]The numbers in bold were used for calculation.

[c]The previous evaluation is made for the food enzyme application EFSA-Q-2021-00066.

[d]The food manufacturing process was not included in the food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023a).

The additional two uses of the food enzyme are described below.

In the production of distilled alcoholic beverages and non-distilled alcoholic beverages other than from grape, the food enzyme is added to fruit, such as apples and pears, during the peeling and the crushing. It is also added to the fruit must before fermentation.[7] The enzymatic treatment contributes to the degradation of pectin that, in turn, can increase the processability and the yield of the fruit must.[8] The food enzyme-TOS is removed by distillation in brandies but remains in other alcoholic beverages like cider and perry.[9]

Based on the thermostability evaluated previously (EFSA CEP Panel, 2023b) and the downstream processing steps applied in the food manufacturing processes, it is expected that the polygalacturonase is inactivated during most of the food manufacturing processes, but may remain active in wine and fruit and vegetable juices, depending on the pasteurisation conditions.

3.1.2 | Dietary exposure estimation

In accordance with the guidance document (EFSA CEP Panel, 2021), dietary exposure was calculated only for the four food manufacturing processes where the food enzyme-TOS remains in the final foods.

Chronic exposure to the food enzyme-TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSA CEP Panel, 2021). The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP Panel, 2023a). Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period (days) and normalised for body weight (bw). This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only 1 day per subject were excluded and high-level exposure/intake was calculated for only those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011).

Table 2 provides an overview of the derived exposure estimates across all surveys. Detailed mean and 95th percentile exposure to the food enzyme-TOS per age class, country and survey, as well as contribution from each FoodEx category to the total dietary exposure are reported in Appendix A – Tables 1 and 2. For the present assessment, food consumption data were available from 48 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 26 European countries (Appendix B). The highest dietary exposure was estimated to be 0.087 mg TOS/kg bw per day in children at the 95th percentile.

[6]Spontaneous data submission February 2024/p. 2.

[7]Spontaneous data submission February 2024/pp. 3–4.

[8]Spontaneous data submission February 2024/pp. 3–4.

[9]Additional data November 2023.

TABLE 2 Updated dietary exposure to the food enzyme–TOS in six population groups.

Population group	Estimated exposure (mg TOS/kg body weight per day)					
	Infants	**Toddlers**	**Children**	**Adolescents**	**Adults**	**The elderly**
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0.001–0.017 (12)	0.006–0.052 (15)	0.002–0.029 (19)	0.001–0.016 (21)	0.002–0.012 (22)	0.002–0.009 (23)
Min–max 95th percentile (number of surveys)	0.005–0.054 (11)	0.026–0.081 (14)	0.006–0.087 (19)	0.004–0.053 (20)	0.009–0.041 (22)	0.008–0.029 (22)

3.1.3 | Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the following sources of uncertainties have been considered and are summarised in Table 3.

TABLE 3 Qualitative evaluation of the influence of uncertainties on the dietary exposure estimate.

Sources of uncertainties	Direction of impact
Model input data	
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/–
Use of data from food consumption surveys of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Possible national differences in categorisation and classification of food	+/–
Model assumptions and factors	
Exposure to food enzyme–TOS always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
Use of recipe fractions to disaggregate FoodEx categories	+/–
Use of technical factors in the exposure model	+/–
Exclusion of three processes from the exposure estimation: – Production of distilled alcoholic beverages – Production of green coffee beans by demucilation – Production of plant extracts as flavouring preparations	–

Abbreviations: +, uncertainty with potential to cause overestimation of exposure; –, uncertainty with potential to cause underestimation of exposure.

The conservative approach applied to estimate the exposure to the food enzyme-TOS, in particular assumptions made on the occurrence and use levels of this specific food enzyme, is likely to have led to an overestimation of the exposure.

The exclusion of three food manufacturing processes from the exposure estimation was based on > 99% of TOS removal. This is not expected to impact on the overall estimate derived.

3.2 | Margin of exposure

In the previous evaluation, the Panel identified a no observed adverse effect level (NOAEL) of 1000 mg TOS/kg bw per day, the highest dose tested, resulting in a margin of exposure (MOE) of at least 11,494 (EFSA CEP Panel, 2023b).

A comparison of the NOAEL with the newly derived exposure estimates of 0.001–0.052 mg TOS/kg bw per day at the mean and from 0.004 to 0.087 mg TOS/kg bw per day at the 95th percentile resulted in a MOE of at least 11,494.

Despite that more uses were considered in the current assessment, the newly derived MOE is the same as that previously calculated. This is due to the revision of food groups and technical factors used for each food manufacturing process (EFSA CEP Panel, 2023a). In addition, dietary surveys have been updated in the EFSA food consumption database.

4 | CONCLUSION

Based on the data provided for the previous evaluation and the revised margin of exposure, the Panel concluded that the food enzyme endo-polygalacturonase produced with the genetically modified *Aspergillus oryzae* strain AR-183 does not give rise to safety concerns under the revised intended conditions of use.

5 | DOCUMENTATION AS PROVIDED TO EFSA

Application for authorisation of a polygalacturonase from a genetically modified strain of *Aspergillus oryzae* in accordance with regulation (EC) no 1331/2008. March 2023. Submitted by AB ENZYMES GmbH.

Additional information. November 2023. Submitted by AB ENZYMES GmbH.

Spontaneous data submission. February 2024. Submitted by AB ENZYMES GmbH.

ABBREVIATIONS

bw	body weight
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
EU	European Union
IUBMB	International Union of Biochemistry and Molecular Biology
MOE	margin of exposure
NOAEL	no observed adverse effect level
RM	raw material
TOS	total organic solids

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-2023-00524

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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., Roos, Y., Cavanna, D., … Chesson, A. (2024). Safety evaluation of an extension of use of the food enzyme endo-polygalacturonase from the genetically modified *Aspergillus oryzae* strain AR-183. *EFSA Journal*, *22*(4), e8701. https://doi.org/10.2903/j.efsa.2024.8701

APPENDIX A

Dietary exposure estimates to the food enzyme–TOS in details

Appendix A can be found in the online version of this output (in the 'Supporting information' section). The file contains two sheets, corresponding to two tables.

Table 1: Average and 95th percentile exposure to the food enzyme–TOS per age class, country and survey.

Table 2: Contribution of food categories to the dietary exposure to the food enzyme–TOS per age class, country and survey.

APPENDIX B

Population groups considered for the exposure assessment

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From 12 weeks on up to and including 11 months of age	Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia, Spain
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Netherlands, Portugal, Republic of North Macedonia[a], Serbia[a], Slovenia, Spain
Children	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Republic of North Macedonia[a], Serbia[a], Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Bosnia and Herzegovina[a], Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Montenegro[a], Netherlands, Portugal, Romania, Serbia[a], Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Bosnia and Herzegovina[a], Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro[a], Netherlands, Portugal, Romania, Serbia[a], Slovenia, Spain, Sweden
The elderly[b]	From 65 years of age and older	Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro[a], Netherlands, Portugal, Romania, Serbia[a], Slovenia, Spain, Sweden[a]

[a]Consumption data from these pre-accession countries are not reported in Table 2 of this opinion; however, they are included in Appendix A for testing purpose.

[b]The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011).