

Safety evaluation of an extension of use of the food enzyme containing cellulase, endo-1,3(4)- β -glucanase and endo-1,4- β -xylanase from the non-genetically modified *Trichoderma reesei* strain AR-256

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Abstract

The food enzyme containing cellulase (EC 3.2.1.4), endo-1,3(4)- β -glucanase (EC 3.2.1.6) and endo-1,4- β -xylanase (EC 3.2.1.8) is produced with the non-genetically modified *Trichoderma reesei* strain AR-256 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 seven food manufacturing processes. Subsequently, the applicant requested to extend its use to include two additional processes. In this assessment, EFSA updated the safety evaluation of this food enzyme when used in a total of nine food manufacturing processes: processing of cereals and other grains for the production of 1) baked products, 2) cereal-based products other than baked, 3) brewed products, 4) starch and gluten fractions, 5) distilled alcohol; processing of fruits and vegetables for the production of 6) wine and wine vinegar, 7) juices, 8) fruit and vegetable products other than juices and 9) fruit-derived distilled alcoholic beverages other than from grape. As the food enzyme–total organic solids (TOS) is removed from or not carried into the final foods in three food manufacturing processes, the dietary exposure to the food enzyme–TOS was estimated only for the remaining six processes. It was up to 4.049 mg TOS/kg body weight (bw) per day in European populations. Using the no observed adverse effect level (NOAEL) reported in the previous opinion (939 mg TOS/kg bw per day), the Panel derived a revised margin of exposure of at least 232. Based on the revised exposure calculation and the outcome of the previous evaluation, the Panel concluded that this food enzyme does not give rise to safety concerns under the revised intended conditions of use.

KEYWORDS

cellulase, EC 3.2.1.4, EC 3.2.1.6, EC 3.2.1.8, endo-1,3(4)- β -glucanase, endo-1,4- β -xylanase, food enzyme, *Trichoderma reesei*

*The previous evaluation is made for the food enzyme application EFSA-Q-2021-00545.

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1 | INTRODUCTION

Article 3 of the Regulation (EC) No 1332/2008¹ 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 1331/2008² 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 on food enzymes.

On 21 February 2023, a new application has been introduced by the applicant “AB ENZYMES GmbH” for an extension of the conditions of use for Cellulase, glucanase, xylanase from a non-genetically modified strain of *Trichoderma reesei* (strain AR-256).

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 conditions of use for the following food enzyme: Cellulase, glucanase, xylanase from a non-genetically modified strain of *Trichoderma reesei* (strain AR-256), 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 cellulase, endo-1,3(4)- β -glucanase and endo-1,4- β -xylanase from the non-genetically modified *Trichoderma reesei* AR-256.

Additional information was requested from the applicant during the assessment process on 17 October 2023 and received on 20 October 2023 (see ‘[Documentation provided to EFSA](#)’).

¹Regulation (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.

²Regulation (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.

³OJ L 354, 31.12.2008, p. 1.

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, 2023) have been followed to evaluate this application.

2.3 | Public consultation

According to Article 32c(2) of Regulation (EC) No 178/2002⁴ 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 12 October to 2 November 2023.⁵ No comments were received.

3 | ASSESSMENT

The food enzyme under application contains three declared activities:

IUBMB nomenclature	Cellulase
Systematic name	1,4-(1,3;1,4)- β -D-glucan-4-glucanohydrolase
Synonyms	carboxymethyl cellulase; β -1-4-glucanase
IUBMB no	3.2.1.4
CAS no	9012-54-8
EINECS no	232-734-4

Cellulases catalyse the hydrolysis of 1-4- β -glycosidic linkages in cellulose and other β -glucans, resulting in the generation of shorter β -D-glucan chains.

IUBMB nomenclature	Endo-1,3(4)- β -glucanase
Systematic name	3-(1 \rightarrow 3;1 \rightarrow 4)- β -D-glucan 3(4)-glucanohydrolase
Synonyms	endo-1,3- β -D-glucanase; laminarinase; laminaranase; β -1,3-glucanase
IUBMB no	3.2.1.6
CAS no	62213-14-3
EINECS no	263-462-4

Endo-1,3(4)- β -glucanases catalyse the hydrolysis of 1,3- or 1-4- β -glycosidic linkages in mixed-linked β -D-glucans, resulting in the generation of partially hydrolysed β -D-glucans.

IUBMB nomenclature	Endo-1,4- β -xylanase
Systematic name	4- β -D-xylan xylanohydrolase
Synonyms	endo-(1 \rightarrow 4)- β -xylan 4-xylanohydrolase; xylanase; β -1,4-xylanase; β -xylanase
IUBMB no	3.2.1.8
CAS no	9025-57-4
EINECS no	232-800-2

Endo-1,4- β -xylanases catalyse the random hydrolysis of 1,4- β -D-xylosidic linkages in xylans (including arabinoxylans), resulting in the generation of (1-4)- β -D-xylan oligosaccharides of different lengths. All aspects concerning the safety of this food enzyme, when used in seven food manufacturing processes, were evaluated in November 2022 (EFSA CEP Panel, 2022).

⁴Regulation (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.

⁵Accessible at <https://connect.efsa.europa.eu/RM/s/publicconsultation2/a0109000009CylC/pc0689>

Following an application for two additional food manufacturing processes, EFSA revised the exposure assessment and updated the safety evaluation of this food enzyme when used in nine food manufacturing processes.

3.1 | Dietary exposure

The current dietary exposure supersedes section 3.5 of the previous evaluation (EFSA CEP Panel, 2022).

3.1.1 | Revised intended use of the food enzyme

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

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

Food manufacturing process ^a	Raw material (RM)	Recommended use level (mg TOS/kg RM) ^b
Processing of cereals and other grains		
• Production of baked products	Flour	4– 15
• Production of cereal-based products other than baked	Flour	4– 15
• Production of brewed products	Cereals	75– 298
• Production of starch and gluten fractions	Cereals	45–90
• Production of distilled alcohol	Cereals	75–186
Processing of fruits and vegetables		
• Production of juices	Fruit and vegetable	22– 90
• Production of wine and wine vinegar	Fruit	22– 75
• Production of fruit and vegetable products other than juices	Fruit and vegetable	22– 90
• Production of distilled alcoholic beverages other than from grape ^c	Fruit and vegetable	22–90

^aThe names have been harmonised by EFSA to the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023).

^bThe numbers in bold were used for calculation.

^cThe food manufacturing process was not included in the (EFSA CEP Panel, 2023).

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

In the production of fruit and vegetable products other than juices, such as jams and fruit puree, the food enzyme is added to the crushed pulp before pasteurisation.⁷ The combined action of these three enzyme activities reduces the viscosity and improves the consistency of fruit preparations. The food enzyme–TOS remains in these products.

In the production of distilled alcoholic beverages other than from grape, the food enzyme is added to fruits during the peeling and crushing. It is also used to treat the fruit mash before the fermentation and distillation steps.^{8,9} The enzymatic treatment increases the processability and distillation efficiency. The food enzyme–TOS is not carried over with the distilled alcoholic beverages.

Based on the thermostability evaluated previously (EFSA CEP Panel, 2022) and the downstream processing steps applied in the food manufacturing processes, it is expected that all three enzyme activities are inactivated during four of the six food manufacturing processes in which the food enzyme–TOS remains. The enzymatic activity may remain in wine as well as in 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 food manufacturing processes where the food enzyme–TOS remains in the final foods: processing of cereals and other grains for the production of 1) baked products, 2) cereal-based products other than baked, 3) brewed products; processing of fruits and vegetables for the production of 4) wine and wine vinegar, 5) juices, 6) fruit and vegetable products other than juices.

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

⁶Technical dossier/Intended use(s) in food and use level(s) (Proposed normal and maximum use levels)/p. 27; Additional data October 2023.

⁷Technical dossier/ Intended use(s) in food and use level(s) (Proposed normal and maximum use levels)/p. 29.

⁸Technical dossier/ Intended use(s) in food and use level(s) (Proposed normal and maximum use levels)/p. 30.

⁹Technical dossier/ Reaction and fate in foods to which the food enzyme is added.

application of technical conversion factors (EFSA CEP Panel, 2023). Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period (days) and normalised for body weight. 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 4.049 mg TOS/kg body weight (bw) per day in children at the 95th percentile.

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)	0.109–1.262 (12)	0.456–2.604 (15)	0.211–1.425 (19)	0.131–0.879 (21)	0.176–0.703 (22)	0.117–0.538 (23)
Min–max 95th percentile (number of surveys)	0.384–2.945 (11)	1.502–3.825 (14)	0.597–4.049 (19)	0.438–2.648 (20)	0.596–2.091 (22)	0.465–1.500 (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 was always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
Use of recipe fractions in disaggregation FoodEx categories	+/-
Use of technical factors in the exposure model	+/-
Exclusion of other processes from the exposure assessment	-
– Production of starch and gluten fractions	
– Production of distilled alcohol	
– Production of distilled alcoholic beverages other than from grape	

+: 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 939 mg TOS/kg bw per day, the highest dose tested (EFSA CEP Panel, 2022).

The comparison of the NOAEL with the derived exposure estimates of 0.109–2.604 mg TOS/kg bw per day at the mean and from 0.384 to 4.049 mg TOS/kg bw per day at the 95th percentile resulted in a revised margin of exposure (MOE) of at least 232.

4 | CONCLUSION

Based on the data provided for the previous evaluation and the revised margin of exposure, the Panel concluded that the food enzyme containing cellulase, endo-1,3(4)- β -glucanase and endo-1,4- β -xylanase produced with the non-genetically *T. reesei* strain AR-256 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 cellulase, glucanase and xylanase from a strain of *Trichoderma reesei* in accordance with regulation (EC) no 1331/2008. February 2023. Submitted by AB Enzymes GmbH.

Additional information. October 2023. Submitted by AB Enzymes GmbH.

ABBREVIATIONS

bw	body weight
CAS	Chemical Abstracts Service
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
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

ACKNOWLEDGEMENTS

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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

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SUPPORTING INFORMATION

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

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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, the Netherlands, Portugal, Republic of North Macedonia*, Serbia*, 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, the Netherlands, Portugal, Republic of North Macedonia*, Serbia*, Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Bosnia and Herzegovina*, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Montenegro*, the Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Bosnia and Herzegovina*, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro*, the Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden
The elderly^a	From 65 years of age and older	Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro*, the Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden

*Consumption data from these pre-accession countries are not reported in Table 3 of this opinion however, they are included in Appendix A for testing purpose.

^aThe 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).