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Safety evaluation 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. The genetic modifications do not give rise to safety concerns. The food enzyme is free from viable cells of the production organism and its DNA. It is intended to be used in five food manufacturing processes: fruit and vegetable processing for juice production, fruit and vegetable processing for products other than juice, production of wine and wine vinegar, production of plant extracts as flavouring preparations and coffee demucilation. Since residual amounts of total organic solids (TOS) are removed by repeated washing or distillation, dietary exposure to the food enzyme TOS from coffee demucilation and from the production of flavouring extracts was considered not necessary. For the remaining three food processes, dietary exposure was estimated to be up to 0.087 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not indicate a safety concern. The systemic toxicity was assessed by a repeated dose 90-day oral toxicity study in rats. The Panel identified a no observed adverse effect level of 1,000 mg TOS/kg bw per day, the highest dose tested, which, when compared with the estimated dietary exposure, resulted in a margin of exposure of at least 11,494. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and two matches with pollen allergens were found. The Panel considered that, under the intended conditions of use, the risk of allergic reactions upon dietary exposure to this food enzyme, particularly in individuals sensitised to pollen allergens, cannot be excluded. Based on the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

Keywords: food enzyme, endo-polygalacturonase, (1→4)-α-D-galacturonan glycanohydrolase, EC 3.2.1.15, polygalacturonase, *Aspergillus oryzae*, genetically modified microorganism

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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 micro-organisms or products thereof including a product obtained by a fermentation process using micro-organisms: (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.

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 European Union (EU) Community 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.

An application has been introduced by the applicant "AB Enzymes GmbH" for the authorisation of the food enzyme Polygalacturonase from a genetically modified strain of *Aspergillus oryzae* (strain AR-183).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008, the Commission has verified that the application falls within the scope of the food enzyme Regulation and contains all the elements required under Chapter II of that Regulation.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessment on the following food enzyme: Polygalacturonase from a genetically modified strain of *A. oryzae* (strain AR-183) in accordance with Article 29 of Regulation (EC) No 178/2002, and Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

[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, 11.3.2011, pp. 15–24.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme polygalacturonase from a genetically modified strain of *A. oryzae* strain AR-183. The dossier was updated on 21 April 2021.

Additional information was requested from the applicant during the assessment process on 23 June 2021, 21 October 2021 and 25 March 2023, and received on 21 September 2021, 18 February 2022 and 24 June 2022 (see 'Documentation provided to EFSA').

Following the reception of additional data by EFSA on 21 September 2021, EFSA requested a clarification teleconference on 05 October 2021, after which the applicant provided additional data on 18 February 2022.

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) as well as in the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) and following the relevant existing guidance documents of EFSA Scientific Committees.

The 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) as well as the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) have been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance with the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021a).

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. The food enzyme under assessment is intended to be used in five food manufacturing processes: fruit and vegetable processing for juice production, fruit and vegetable processing for products other than juice, production of wine and wine vinegar, production of plant extracts as flavouring preparations and coffee demucilation.

3.1. Source of the food enzyme

The endo-polygalacturonase is produced with the genetically modified filamentous fungus *Aspergillus oryzae* strain AR-183, which is deposited at the Westerdijk Fungal Biodiversity Institute (the Netherlands), with deposit number [redacted].[4] The production strain was identified as *A. oryzae* by [redacted]

[4] Technical dossier/2nd submission/Annex I-7.
[5] Technical dossier/Additional data September 2021/Annex 1.

3.1.1. Characteristics of the parental and recipient microorganisms

The parental strain is [redacted]

3.1.2. Characteristics of introduced sequences

The sequence encoding the endo-polygalacturonase [redacted][9].

3.1.3. Description of the genetic modification process

The purpose of genetic modification was to enable the production strain to synthesise endo-polygalacturonase [redacted]

3.1.4. Safety aspects of the genetic modification

The technical dossier contains all necessary information on the recipient microorganism, the donor organism and the genetic modification process.

The production strain *A. oryzae* strain AR-183 differs from the recipient strain [redacted]

No issues of concern arising from the genetic modifications were identified by the Panel.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004,[11] with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[12]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, [redacted] fermentation system with conventional process controls in place. After completion of the fermentation the solid biomass is removed from the fermentation broth by filtration, leaving a supernatant containing the food enzyme. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in which enzyme protein is retained, while most of the low molecular mass material passes the filtration membrane and is discarded.[13] 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.[14]

[6] Technical dossier/2nd submission/Volume II/p. 2.
[7] Technical dossier/2nd submission/Annex II-1.
[8] Technical dossier/2nd submission/ Volume II/p. 7–8.
[9] Technical dossier/2nd submission/Annex II-4.
[10] Technical dossier/2nd submission/Annex II-6.
[11] 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.
[12] Technical dossier/2nd submission/Annex 8.
[13] Technical dossier/2nd submission/pp. 17-26/Annexes: 10, 11, 12.
[14] Technical dossier/2nd submission/pp. 18, 20/Annex 9.

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 endo-polygalacturonase is a single polypeptide chain of [redacted] amino acids.[15] The molecular mass of the mature protein, calculated from the amino acid sequence, is [redacted] kDa. The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis (SDS–PAGE). A consistent protein pattern was observed across all batches. The gel showed the target protein migrating between the marker proteins of [redacted] kDa in all batches consistent with the calculated mass of the endo-polygalacturonase.[16] The food enzyme was analysed for the presence of cellulase, protease and amylase activities. Cellulase and protease activities were detected.[17] No other enzyme activities were reported.

The in-house determination of endo-polygalacturonase activity is based on the reduction in viscosity of a pectin solution (reaction conditions: [redacted]). The enzymatic activity is calculated relative to the viscosity of the untreated substrate and is expressed in Polygalacturonase Units, PGU/mg determined in comparison to an internal standard of known activity.

The food enzyme has a temperature optimum around [redacted] and a pH optimum around pH [redacted] Thermostability was tested after a pre-incubation of the food enzyme at 85°C for different periods (pH 3.9). Endo-polygalacturonase activity decreased by more than 99% after 1 min of pre-incubation and was not detected after 4 min.[18]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches of the food enzyme (Table 1) used for commercialisation, one of which (batch 1) was also used for the toxicological studies. The mean total organic solids (TOS) of the three food enzyme batches is 14.9% and the mean enzyme activity/TOS ratio is 5,278,590 PGU/mg TOS.[19]

Table 1: Composition of the food enzyme

Parameters	Unit	1[(a)]	2	3
Endo-polygalacturonase activity	PGU/mg batch[(b)]	720,500	856,000	775,000
Protein	%	11.6	12.6	11.5
Ash	%	0.4	0.4	0.4
Water	%	84.3	84.5	85.4
Total organic solids (TOS)[(c)]	%	15.3	15.1	14.2
Activity/TOS	PGU/mg TOS	4,709,150	5,668,874	5,457,746

(a): Batch used for the toxicological studies.
(b): PGU: Polygalacturonase Unit (see Section 3.3.1).
(c): TOS calculated as 100% – % water – % ash.

3.3.3. Purity

The lead content in the three 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, the levels of arsenic, cadmium and mercury were below the limits of detection (LODs) of the employed methods.[20,21]

[15] Technical dossier/Additional data September 2021.
[16] Technical dossier/2nd submission/pp. 9/Annex 1.
[17] Technical dossier/2nd submission/pp. 14/Annex 3.
[18] Technical dossier/2nd submission/pp. 12-14/Annex 6.
[19] Technical dossier/2nd submission/pp. 11, 40/Annexes: 3, 13, 14, 15.
[20] LoDs: Pb, Cd, Hg = 0.025 mg/kg; As = 0.25 mg/kg.
[21] Technical dossier/2nd submission/pp. 11-12/Annexes: 3, 4.

The food enzyme 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).[21] No antimicrobial activity was detected in any of the tested batches.[21]

Strains of *Aspergillus*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Frisvad et al., 2018). The presence of aflatoxins: B1, B2, G1, G2, fumonisins: B1, B2, ochratoxin A and sterigmatocystin was examined in the three food enzyme batches. All were below the LoD of the applied method.[21,22] Adverse effects caused by the possible presence of other secondary metabolites are addressed by the toxicological examination of the food enzyme–TOS.

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

3.3.4. Viable cells and DNA of the production strain

The absence of viable cells of the production strain in the food enzyme was demonstrated in three independent batches of the food enzyme analysed in quadruplicate. [redacted][23] No colonies were produced.[24]

The absence of recombinant DNA in the food enzyme was demonstrated by polymerase chain reaction (PCR) analysis of three batches of the final enzyme product in triplicate. No DNA was detected with primers that would amplify a [redacted][25]

3.4. Toxicological data

A battery of toxicological tests, including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian cell micronucleus assay and a repeated dose 90-day oral toxicity study in rats, has been provided. The commercial batch 1 (Table 1) was used for these studies.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was performed according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997) and following Good Laboratory Practice (GLP).[26]

Five strains of *Salmonella* Typhimurium (TA98, TA100, TA1535, TA1537 and TA102) were used in the presence or absence of metabolic activation (S9-mix), applying the standard plate incorporation method (Experiment I) and pre-incubation method (Experiment II). The experiments were carried out in triplicate using six different concentrations of the food enzyme (31.6, 100, 316, 1,000, 2,500 and 5,000 μg TOS/plate).

No cytotoxicity was observed at any concentration level of the test substance. Upon treatment with the food enzyme, there was no significant increase in revertant colony numbers above the control values in any strain with or without S9-mix.

The Panel concluded that the food enzyme did not induce gene mutations under the test conditions employed in this study.

3.4.1.2. *In vitro* mammalian cell micronucleus assay

The *in vitro* micronucleus test was carried out according to OECD Draft Guideline 487 (OECD, 2016) and following GLP.[27]

A pre-experiment for cytotoxicity was performed at concentrations ranging from 7.8 to 5,000 μg TOS/mL. Based on these results, two separate experiments were performed in duplicate cultures with human peripheral whole blood lymphocytes. In the first experiment, for the short-term treatment (4 h followed by 40 h recovery period), the cells were exposed to the food enzyme at 400, 500 and 600 μg

[22] LoDs: aflatoxins B1, B2, G1, G2 = 0.1 μg/kg each; fumonisins B1, B2 = 20 μg/kg each; ochratoxin A = 0.5 μg/kg; sterigmatocystin = 10 μg/kg.
[23] Technical dossier/2nd submission/Annex II-9.
[24] Technical dossier/2nd submission/Annex II-8.
[25] Technical dossier/2nd submission/Annex II-10.
[26] Technical dossier/Annex 13.
[27] Technical dossier/Annex 14.

TOS/mL without metabolic activation (S9-mix) and at 250, 500 and 750 μg TOS/mL with S9-mix. In the second experiment, the cells were exposed to the food enzyme at 25, 50 and 100 μg TOS/mL in the continuous treatment (44 h) in the absence of S9-mix.

The highest level of cytotoxicity (55%) was observed in the continuous treatment at 100 μg TOS/mL. A statistically significant increase in the frequency of binucleated cells with micronuclei (MNBNs) was observed at the highest concentration tested (500 μg TOS/mL) in the short-term treatment in the presence of S9-mix. The value was within the 95% of the laboratory historical control range, not concentration-dependent and was considered not biologically relevant. The frequency of MNBNs was comparable to the negative controls at all the other concentrations and conditions of treatment.

The Panel concluded that the food enzyme endo-polygalacturonase did not induce an increase in the frequency of MNBNs in cultured human peripheral blood lymphocytes, under the test conditions employed in this study.

3.4.2. Repeated dose 90-day oral toxicity study in rodents

The repeated dose 90-day oral toxicity study followed the OECD Test Guideline 408 (OECD, 2018) and GLP.[28] The authors reported the following deviations: the functional observations in week 13 were performed only on five females from the control, low-, mid- and high-dose groups and the same animals were unintentionally not fasted before blood sampling for haematological and clinical chemistry analyses. The Panel considered that these deviations did not impact the evaluation of the study. Groups of 10 male and 10 female Wistar (Crl:WI(Han)) rats received by gavage the food enzyme in 100, 300 and 1,000 mg TOS/kg bw per day. Controls received the vehicle (water).

No mortality was observed.

During the weekly detailed clinical observation, the following statistically significant signs were recorded in females: an increase in sleeping (low-, mid- and high-dose groups: +67%) and a decreased moving in the cage (low-, mid- and high-dose groups: −100%) in week 1, a decrease in sleeping (low- and mid-dose groups: −70% and −50%, respectively) and an increased moving in the cage in low- and mid-dose groups in week 5, the changes in skin in the mid-dose group in week 11 and a statistically significantly increased response to handling in mid- (+13%) and high-dose (+13%) groups in week 12. The Panel considered these changes as not toxicologically relevant as they were only observed in one sex, they were only observed sporadically and there was no dose–response relationship.

The functional observation battery tests performed in week 13 showed a statistically significantly increased moving in the cage in all treated males (scores: C: 0, low dose: 1, mid dose 0.8, high dose: 0.9) and a statistically significant decrease in the observation of fear in low- and mid-dose females (−29 and −43%, respectively). The Panel considered the changes as not toxicologically relevant as they were only observed sporadically (both parameters), they were only observed in one sex (both parameters) and there was no dose–response relationship (both parameters).

Haematological investigations revealed a statistically significantly lower mean value for white blood cells (WBC, −26.12%) in low-dose males. The Panel considered this change as not toxicologically relevant because there was no dose–response relationship and it was only observed in one sex.

The clinical chemistry investigations revealed a statistically significant decrease in alanine aminotransferase (ALT) in the high-dose male group (−25%), in aspartate aminotransferase (AST) in mid- and high-dose male groups (−23% and −34%, respectively) and in sodium in the high-dose male group (−4%). In females, a statistically significantly increased total bilirubin was recorded in the high-dose group (+33%). The Panel considered these changes as not toxicologically relevant as the changes were small (ALT, AST, Na), they were only observed in one sex (all parameters), there were no changes in other relevant parameters (bilirubin and red blood cells count and alkaline phosphatase) and there were no histopathological changes in the liver related to the increase in bilirubin.

No other statistically significant or biologically relevant differences to controls were reported.

The Panel identified the no observed adverse effect level (NOAEL) of 1,000 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity

The allergenicity assessment considers only the food enzyme and not any carrier or other excipient, which may be used in the final formulation.

[28] Technical dossier/Annex 15.

The potential allergenicity of the endo-polygalacturonase produced with the genetically modified *A. oryzae* strain AR-183 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, two matches were found. The matching allergens were profilin Zea m 13 produced by *Zea mays* (Maize) and glycosyl hydrolase produced by *Sorghum halepense* (Johnson grass), both pollen allergens.[29]

No information was available on oral and respiratory sensitisation or elicitation reactions of this endo-polygalacturonase.

The Panel noted that oral allergy syndrome (OAS) is associated with sensitisation to many pollen allergens, such as that from Johnson grass (Ibarolla et al., 2004; Chiang et al., 2006), and maize (Jimenez-Lopez et al., 2012).

However, in case of OAS, the inflammation is usually restricted to the buccal cavity since the allergens are rapidly degraded by gastric enzymes upon ingestion and seldomly leads to anaphylaxis (Sarkar et al., 2018).

[redacted], a product that may cause allergies or intolerances (listed in the Regulation (EU) No 1169/2011[30]), is used as a raw material. In addition, [redacted], a known source of allergens, is also present in the media fed to the microorganisms. However, during the fermentation process, these products will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the fungal biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that no potentially allergenic residues are present in the food enzyme.

The Panel considered that, under the intended conditions of use, the risk of allergic reactions upon dietary exposure to this food enzyme, particularly in individuals sensitised to pollen allergens, cannot be excluded.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in five food processes at the recommended use levels summarised in Table 2.

Table 2: Intended uses and recommended use levels of the food enzyme as provided by the applicant[31]

Food manufacturing process[(a)]	Raw material (RM)	Maximum recommended use level (mg TOS/kg RM)[(b)]
Fruit and vegetable processing for juice production	Fruits and vegetables	**2**
Fruit and vegetable processing for products other than juice	Fruits and vegetables	**1**
Production of wine and wine vinegar	Grapes	**2**
Production of plant extracts as flavouring preparations[32]	Fruit and vegetables	1.5
Coffee demucilation	Coffee cherry	0.5

(a): The description has been harmonised by EFSA according to the 'EC working document describing the food processes in which food enzymes are intended to be used' – not yet published at the time of adoption of this opinion.
(b): The numbers in bold were used for calculation.

[29] Technical dossier/2nd submission/pp. 41-43/Annex 2.
[30] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.
[31] Technical dossier/2nd submission/p. 30.
[32] Additional data September 2021/Answer 6.

In fruit and vegetable processing for juice production, the function of the endo-polygalacturonase is to hydrolyse galacturonan-rich cell wall components (e.g. pectin) in different raw materials at various points in the production process. For juice production, the food enzyme can be added during the peeling and crushing, to the crush mash of fruits/vegetables (with or without peels) and/or to the pressed juice before clarification and filtration.[33] The disruption of the gel structure reduces the viscosity, thus improving the pressing ability of the pulp and consequently increasing the yield of fruit juices. The enzymatic treatment can reduce haze and enhance colour and aroma.[34] The food enzyme-TOS remains in the juices.

For the production of other fruit and vegetable products such as puree, the endo-polygalacturonase is added to the crushed pulp before pasteurisation.[35] The enzymatic treatment reduces viscosity and improves the consistency of puree.[36] The food enzyme-TOS remains in these products.

In wine and wine vinegar production, endo-polygalacturonase is often added together with other cell wall hydrolytic enzymes during crushing. It can be added also during maceration and clarification steps.[37] Such enzymatic treatment aids pressing and facilitates the extraction of aromatic compounds. The food enzyme-TOS may remain in wine and wine vinegar.

To produce essential oils, fruit components rich in oil are treated with the endo-polygalacturonase to assist the release of aromatic compounds from the raw material. It is expected that the food enzyme TOS partitions with the water phase, therefore, is not carried into the oil phase.[32] These aroma concentrates are primarily used in the reconstitution of juices.

Samples of the apple aroma concentrate and orange aroma oil and additional samples obtained by trichloroacetic acid precipitation were separated by SDS–PAGE and stained with Coomassie Blue. No proteins of the food enzyme were detected.[38,39] The Panel accepted that this evidence as sufficient to support the lack of TOS transfer into the essential oils.

In coffee processing, endo-polygalacturonase is added to the green coffee berry during pulping and fermentation to degrade the mucilage.[40] The food enzyme-TOS is removed during the subsequent washing steps (EFSA CEP Panel, 2021b).

Based on data provided on thermostability (see Section 3.3.1), it is expected that the endo-polygalacturonase is inactivated during all the above-mentioned food processes.

3.5.2. Dietary exposure estimation

In accordance with the guidance document (EFSA CEP Panel, 2021a), a dietary exposure was calculated only for food manufacturing processes where the food enzyme-TOS remains in the final foods, i.e., fruit and vegetable processing for juice production, fruit and vegetable processing for products other than juice, and the production of wine and wine vinegar.

Chronic exposure to the food enzyme-TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSA CEP Panel, 2021a). The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP Panel, 2021b). 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 3 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 43 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in

[33] Technical dossier/2nd submission/p. 31.
[34] Additional data February 2022.
[35] Technical dossier/2nd submission/p. 32.
[36] Technical dossier/2nd submission/pg. 32.
[37] Technical dossier/2nd submission/p. 33.
[38] Additional data February 2022/Annex 1.
[39] Additional data June 2022/Annex 1.
[40] Technical dossier/2nd submission/p. 34.

22 European countries (Appendix B). The highest dietary exposure was estimated to be 0.087 mg TOS/kg bw per day in children of 3–9 years of age at the 95th percentile.

Table 3: Summary of estimated dietary exposure to 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)	0.001–0.017 (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)

3.5.3. 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 4.

Table 4: 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 to disaggregate FoodEx categories	+/–
Use of technical factors in the exposure model	+/–
Exclusion of other processes from the exposure assessment – Coffee demucilation – Production of plant extract as flavouring preparations	–

+: Uncertainty with potential to cause overestimation of exposure.
–: Uncertainty with potential to cause underestimation of exposure.

The conservative approach applied to the exposure estimate to 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 two food manufacturing processes from the exposure assessment was based on > 99% of TOS removal during these processes and is not expected to have an impact on the overall estimate derived.

3.6. Margin of exposure

A comparison of the NOAEL (1,000 mg TOS/kg bw per day) from the 90-day rat study with the 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 margin of exposure of at least 11,494.

4. Conclusions

Based on the data provided, the removal of TOS during coffee demucilation and production of plant extracts as flavouring preparations and the derived margin of exposure for the remaining three food manufacturing processes, the Panel concluded that the food enzyme endo-polygalacturonase produced with the genetically modified *A. oryzae* strain AR-183 does not give rise to safety concerns under the intended conditions of use.

The CEP Panel considered the food enzyme free from viable cells of the production organism and recombinant DNA.

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. February 2021. Submitted by AB ENZYMES GmbH.

Additional information. September 2021, February 2022 and June 2022. Submitted by AB ENZYMES GmbH.

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Abbreviations

ALT	alanine aminotransferase
AST	aspartate aminotransferase
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
FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practice
GMM	genetically modified microorganism
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LoD	limit of detection
MoE	margin of exposure
NOAEL	no observed adverse effect level
OAS	oral allergy syndrome
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	total organic solids
WBC	white blood cells
WHO	World Health Organization

Appendix A – Dietary exposure estimates to the food enzyme–TOS in details

Information provided in this appendix is shown in an excel file (downloadable at https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2023.7836#support-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, 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, Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, 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, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden

(a): 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).