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Safety evaluation of the food enzyme pectin lyase from the genetically modified *Trichoderma reesei* strain RF6199

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Abstract

The food enzyme pectin lyase ((1-4)-6-O-methyl-α-D-galacturonan lyase; EC 4.2.2.10) is produced with the genetically modified *Trichoderma reesei* strain RF6199 by AB Enzymes GmbH. The genetic modifications do not give rise to safety concerns. The food enzyme is considered free from viable cells of the production organism and its DNA. It is intended to be used in six manufacturing processes: fruit and vegetable processing for juice production, fruit and vegetable processing for fruit brandies, fruit and vegetable processing for products other than juices, wine and wine vinegar production, refined and unrefined sugar production and coffee bean demucilation. Foods obtained from fruit processing for fruit brandies and coffee bean demucilation, as well as refined sugars, were excluded from dietary exposure estimation. For the remaining four processes, dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.2 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 means of 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, results in a margin of exposure of at least 5,000. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and no match was found. The Panel considered that, under the intended conditions of use, the risk of allergic reactions by dietary exposure cannot be excluded, but the likelihood of such reactions is low. 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, pectin lyase, (1-4)-6-O-methyl-α-D-galacturonan lyase, EC 4.2.2.10, pectin trans-eliminase, *Trichoderma reesei*, genetically modified microorganism

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1. Introduction

Article 3 of the Regulation (EC) No 1332/2008[1] provides definitions 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.

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 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.

Three applications have been introduced by the companies "Paninkret Chem. Pharm. Werk GmbH" and "AB Enzymes GmbH" for the authorisation of the food enzymes trypsin and chymotrypsin from pig pancreas, pectin lyase from a genetically modified strain of *Trichoderma reesei* (strain RF6199) and endo 1,4-beta xylanase from a genetically modified strain of *Aspergillus acidus* (strain RF7398).

Following the requirements of Article 12.1 of Commission Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008, the Commission has verified that the three applications fall within the scope of the food enzyme Regulation and contain 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 food enzymes trypsin and chymotrypsin from pig pancreas, pectin lyase from a genetically modified strain of *Trichoderma reesei* (strain RF6199) and endo 1,4-beta xylanase from a

[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.

genetically modified strain of *Aspergillus acidus* (strain RF7398) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. Interpretation of the terms of reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of food enzyme pectin lyase from genetically modified *Trichoderma reesei* strain RF6199.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme pectin lyase from the genetically modified strain of *Trichoderma reesei* strain RF 6199. The dossier was submitted on 2 December 2013.

Additional information was requested from the applicant during the assessment process on 8 June 2021, and on 24 November 2021, and was consequently provided (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, 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 current 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) has been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance to the methodology described in the CEF Panel 'Statement on the exposure assessment of food enzymes' (EFSA CEF Panel, 2016).

3. Assessment

IUBMB nomenclature	Pectin lyase
Systematic name	(1-4)-6-O-methyl-α-D-galacturonan lyase
Synonyms	Pectin trans-eliminase, polymethylgalacturonic transeliminase, pectin methyltranseliminase
IUBMB No	4.2.2.10
CAS No	9033-35-6
EINECS No	232-894-5

Pectin lyases catalyse a β-eliminative cleavage of 1,4-α-D-galactosiduronic linkages in galacturonans, to produce oligosaccharides with 4-deoxy-6-*O*-methyl-α-D-galact-4-enuronosyl groups at their non-reducing ends. The food enzyme is intended to be used in six food manufacturing processes: fruit and vegetable processing for juice production, for fruit brandies and for products other than juices (purees only), wine and wine vinegar production, refined and unrefined sugar production and coffee bean demucilation.

3.1. Source of the food enzyme

The pectin lyase is produced with *T. reesei* strain RF6199, which is deposited in the Westerdijk Fungal Biodiversity Institute (CBS) with the deposit number [redacted].[4] The production strain was identified as *T. reesei* by [redacted].[5]

[4] Technical dossier/Confidential/Annex 7.
[5] Technical dossier/Additional information June 2022/Annex 2.

3.1.1. Characteristics of the parental and recipient microorganisms

The strain *T. reesei* [redacted] (Seidl et al., 2008; Peterson and Nevalainen, 2012) [redacted].[6]

The recipient strain, [redacted].[7]

3.1.2. Characteristics of the introduced sequences

[redacted].[8]

3.1.3. Description of the genetic modification process

The purpose of genetic modification [redacted].[9]

[redacted].[10]

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 *T. reesei* RF6199 differs from the recipient strain [redacted] in its capacity to produce the pectin lyase [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, fed-batch 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 filtrate

[6] Technical Dossier/1st submission/Volume III/Appendix 2.
[7] Technical Dossier/1st submission/Volume III/Appendix 1.
[8] Technical Dossier/1st submission/Volume III/Appendix 7.
[9] Technical Dossier/1st submission/Volume III/Appendix 10.
[10] Technical Dossier/1st submission/Volume III/Appendix 12.
[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/1st submission/Volume II/Annex 9.

containing the food enzyme. The filtrate 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. Finally, the food enzyme is spray-dried prior to analysis.[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]

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 pectin lyase is a single polypeptide chain of [redacted] amino acids.[15] The molecular mass of the mature protein, calculated from the amino acid sequence, was [redacted] kDa.[15] The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). A consistent protein pattern was observed across all batches. The gels showed two major protein bands corresponding to molecular masses of about [redacted] and [redacted] kDa (glycosylated forms).[16] Cellulase, β-glucanase and xylanase activities were reported to be present in the food enzyme.[17]

The in-house determination of enzyme activity is based on the cleavage of citrus pectin (reaction conditions: pH 5.8, 30°C, 8 min). The enzymatic activity is determined by measuring the release of oligosaccharides with 4-deoxy-6-*O*-methyl-α-D-galact-4-enuronosyl groups at their non-reducing ends, spectrophotometrically at 235 nm. Enzyme activity is expressed in Pectin transeliminase units (PTF). One PTF unit is defined as the amount of enzyme that increases the absorbance at 235 nm by 0.01 per minute under the assay conditions.[18]

The food enzyme has a temperature optimum around 40°C (pH 5.8) and around 55°C (pH 3.5), and a pH optimum around pH 5.0 (30°C). Thermostability was tested after pre-incubation at 85°C (pH 4.5) for various periods. The enzyme activity decreased by 76% after 1 min of pre-incubation, showing no residual activity after 8 min of pre-incubation.[19]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation and one batch produced for the toxicological tests (Table 1).[20] The mean total organic solids (TOS) of the three batches for commercialisation was 20.7% and the mean enzyme activity/mg TOS ratio 8,664 PTF/mg TOS.

Table 1: Composition of the food enzyme

Parameters	Unit	Batches			
		1	2	3	4[(a)]
Pectin lyase activity	PTF/mg[(b)]	1,650	1,550	2,180	12,200
Protein	%	17.9	14.0	17.6	71.1
Ash	%	0.2	0.6	0.2	0.9
Water	%	77.3	81.5	78.0	5.7
Total organic solids (TOS)[(c)]	%	22.5	17.9	21.8	93.4
Pectin lyase activity/TOS	PTF/mg TOS	7,333	8,659	10,000	13,062

(a): Batch used for the toxicological tests.
(b): PTF: Pectin transeliminase units (see Section 3.3.1).
(c): TOS calculated as 100% – % water – % ash.

[13] Technical Dossier/1st submission/Volume I/pg. 40–49.
[14] Technical Dossier/1st submission/Volume II/Annexes: 12, 14; Additional information April 2015.
[15] Technical Dossier/Additional information April 2015.
[16] Technical Dossier/1st submission/Volume I/pg. 31; Volume II/Annex 4; Additional information April 2015.
[17] Technical Dossier/1st submission/Volume I/pg. 29, 33.
[18] Technical Dossier/1st submission/Volume I/pg. 31–32; Volume II/Annex 3.
[19] Technical Dossier/1st submission/Volume I/pg. 33–34; Volume II/Annex 6.
[20] Technical Dossier/Additional information June 2022/Annex 3.

3.3.3. Purity

The lead content in the three commercial batches was below 0.05 mg/kg,[21] 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, mercury and cadmium were below the limits of detection (LoD) of the employed methods.[22,23]

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 tested batches.[21]

Strains of *Trichoderma*, 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 and G2, ochratoxin A, fumonisins B1 and B2, zearalenone, deoxinivalenol, T-2 toxin, HT2-toxin and sterigmatocystin was examined in the four food enzyme batches and all were below the LoD of the applied methods.[21,24] Adverse effects caused by the possible presence of other secondary metabolites is 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 [redacted]. No colonies of the production strain were detected. A positive control was included.[25]

The absence of recombinant DNA in the food enzyme [redacted].[26]

3.4. Toxicological data

A battery of toxicological tests, including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian chromosomal aberration test and a repeated dose 90-day oral toxicity study in rats, has been provided. The toxicological assays were performed with batch 4 (Table 1), which was produced in a pilot scale. It had similar protein pattern and chemical purity as the batches used for commercialisation and was considered suitable as a test item by the Panel.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was performed according to the Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997a) and following good laboratory practice (GLP).[27]

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 (the first experiment) and pre-incubation method (the second experiment). The experiments were carried out in triplicate, using eight and six concentrations of the food enzyme: 3, 10, 33, 100, 333, 1,000, 2,500 and 5,000 μg TOS/plate and 33, 100, 333, 1,000, 2,500 and 5,000 μg TOS/plate, respectively.

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.

[21] Technical Dossier/1st submission/Volume I/pg. 30; Volume II/Annex 2; Additional information April 2015/New Annex 1.
[22] Technical Dossier/Additional information September 2021/Annexes 2 and 7.
[23] LoDs: Pb, Cd = 0.05 mg/kg each; Hg = 0.1 mg/kg; As = 0.5 mg/kg.
[24] LoDs: aflatoxins (B1, B2, G1, G2) = 0.05 μg/kg each; fumonisin (B1, B2) = 10 μg/kg; ochratoxin A = 2 μg/kg; T-2 toxin = 20 μg/kg; HT2-toxin = 20 μg/kg; zearalenone = 10 μg/kg; deoxynivalenol = 50 μg/kg; stergimatocystin = 10 μg/kg.
[25] Technical Dossier/Additional information June 2022/Annex 1.
[26] Technical Dossier/Additional information September 2021/Annex 3.
[27] Technical dossier/Annex 17.

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 chromosomal aberration test

The *in vitro* mammalian chromosomal aberration test was carried out in Chinese hamster V79 lung cells according to OECD Test Guideline 473 (OECD, 1997b) and following GLP.[28]

Based on the results of a range-finding study, two separate experiments were carried out in duplicate with and without metabolic activation (S9-mix). In the first experiment, the cells were exposed to the food enzyme at 3,567.3, 4,459.2 and 5,351 μg/mL, corresponding to 3,333, 4,167 and 5,000 μg TOS/mL, in the short-term treatment (4 h followed by 14 h recovery period) with and without S9-mix. In the second experiment, the cells were exposed to the food enzyme at 2,675.5, 4,013.3 and 5,351 μg/mL, corresponding to 2,500, 3,750 and 5,000 μg TOS/mL, in the continuous treatment (18 h) in the absence of S9-mix.

No reduction of the mitotic index was observed at any concentration. A statistically significant increase in structural chromosomal aberrations (4% vs. 1% in the control) was observed at 2,675.5 μg/mL (corresponding to 2,500 μg TOS/mL) in the continuous treatment. However, this value was within the laboratory historical control data range (0.0%–4.0%) and was not considered biologically relevant. The frequency of structural and numerical chromosomal aberrations in treated cultures was comparable to the values detected in negative controls and within the range of the laboratory historical control data at all the other concentrations and conditions of treatment.

The Panel concluded that food enzyme did not induce chromosome aberrations under the test conditions employed for this study.

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

The repeated dose 90-day oral toxicity study was performed in accordance with the OECD Test Guideline 408 (OECD, 1998) and following GLP.[29]

Groups of 10 male and 10 female SPF-bred Wistar rats (RccHan™:WIST) received by gavage the food enzyme at 100, 300 and 1,000 mg TOS/kg bw per day for 91/92 days. Controls received the vehicle (distilled water).

No mortality was observed.

The feed consumption was statistically significantly increased on days 43–50 and 50–57 in low-dose males (+6% and +7%, respectively) and decreased on days 43–50 in high-dose males (−6%). The relative feed consumption (g/kg bw per day) was statistically significantly decreased on days 22–29, 43–50, 50–57, 57–64, 71–78, 78–85 and 85–91 in mid-dose females (−7%, −10%, −8%, −9%, −10%, −8% and −13%, respectively). The Panel considered the changes as not toxicologically relevant, as they were only recorded sporadically, they were only observed in one sex, there was no dose–response relationship (except the feed consumption in males on days 43–50), there was no statistically significant change in the overall mean feed consumption and relative feed consumption over the treatment, and there were no changes in the body weight and body weight gain.

In the functional observations, a statistically significant decrease in locomotor activity was observed in a 50- to 60-min interval and in the overall 0- to 60-min interval in mid-dose males (−75% and −28%, respectively), and for 30- to 40-min and 50- to 60-min intervals in high-dose males (−62% and −83%, respectively). The Panel considered the changes as not toxicologically relevant as they were only recorded sporadically, they were only observed in one sex and there was no dose–response relationship (0- to 60-min interval).

The haematological investigation revealed a statistically significantly lower red cell volume distribution width (RDW) (−12%) and a prolonged activated partial thromboplastin time (PTT) (+19%) in high-dose females. The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (both parameters), there was no changes in the other relevant parameters (for RDW in red blood cell count and in mean corpuscular volume, for PTT in prothrombin time) and the values (both parameters) were within the ranges of the historical control data.

The clinical chemistry investigation revealed a statistically significant decrease in creatine kinase (CK) activity in high-dose males (−38%), an increase in sodium (Na) levels in mid- and high-dose males (+1% in each group), an increase in chloride (Cl) levels in mid-dose males (+1.6%), a decrease

[28] Technical dossier/Annex 18.
[29] Technical dossier/Annex 20.

in lactate dehydrogenase activity (LDH) (−35%) in mid-dose females, an increase in phosphorus (P) level (+16%) in mid-dose females. The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (all parameters), there was no dose–response relationship (Na, Cl, LDH, P), the changes were small (CK, LDH, Na, Cl) and the changes were within the historical control values.

Statistically significant changes in organ weight included increase in the absolute adrenal weight (+16%) and in the adrenal to-brain weight ratio (+16%) in low-dose males. The Panel considered the changes as not toxicologically relevant as there was no dose–response relationship, the changes were only observed in one sex and there were no histopathological changes in the adrenals.

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

The Panel identified a 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 considered only the food enzyme and not any carrier or other excipient, which may be used in the final formulation.

The potential allergenicity of the pectin lyase produced with the genetically modified *T. reesei* strain RF6199 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 match was found.[30]

No information was available on oral and respiratory sensitisation or elicitation reactions of this pectin lyase.

Pectate lysases are allergens often present in pollen, such as in cedar trees. This may explain the high degree of cross-reactivity of lyases with cedar pollen allergens (Terumi Midoro-Horiuti et al., 2003). The oral allergy syndrome, i.e. allergic reactions mainly in the mouth, associated with sensitisation to cedar pollen seldom leads to severe systemic anaphylaxis. Since this pectin lyase showed no homology with known allergens (including those from cedar pollen), the Panel expected no allergic reactions in individuals sensitised to cedar pollen.

[redacted], a product that may cause allergies or intolerances (listed in the Regulation (EU) No 1169/2011[31]), is used as raw material.[32] 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 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 cannot be excluded, but the likelihood of such reactions is low.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

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

[30] Technical Dossier/1st submission/Volume I/pg. 64–66; Volume II/Annex 21.

[31] 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.

[32] Technical Dossier/Additional information September 2021.

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

Food manufacturing process[(a)]	Raw material (RM)	Recommended dosage of the food enzyme (average – maximum) (mg TOS/kg RM)[(b)]
Fruit and vegetable processing for juices production	Fruits or vegetables	0.06–**3**
Fruit and vegetable processing for fruit brandies	Fruits or vegetables	0.3–0.6
Fruit and vegetable processing for products other than juices (purees only[(d)])	Fruits	0.2–**0.5**
Wine and wine vinegar production	Grapes	0.15–**1.5**
Refined and unrefined sugar production	Sliced sugar beets	1.2–**4.1**
Coffee bean demucilation	Coffee cherries	3

(a): The description provided by the applicant 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.
(c): Technical dossier/p. 55 and Additional information September 2021/Response to questions 9 and 10 and Additional information June 2022/Response to questions 4 and 5.
(d): Additional information September 2021/Response to question 9.

This food enzyme is used to treat fruit and vegetable materials to produce a wide range of foods as consumed and food ingredients. Pectin lyases cleave pectins present in the cell wall. The enzymatic treatment decreases viscosity, thereby easing pressing and releasing of cell contents. To produce juices, pectin lyase is added to crushed mash of fruits/vegetables, or added to the crude juices for depectinisation.[33] This food enzyme is intended for the production of all types of juices, such as ready-to-drink juices, concentrated juices, dehydrated juices, nectars, etc.[34] The food enzyme–TOS remains in juices.

The pectin-lyase-treated fruit and vegetable mash can be directly subject to fermentation and distillation to obtain fruit brandies.[35] The food enzyme–TOS is not carried over with the distilled alcohols (EFSA CEP Panel, 2021).

To produce food products other than juices, the applicant specified that this food enzyme will be used only to produce purees.[36] The food enzyme–TOS remains in purees.

In wine production, the food enzyme is added during maceration before pressing and to the must.[37] Pectin lyases disrupt the cell walls, resulting in a higher yield and the release of flavouring substances. The food enzyme–TOS remains in wines.

To produce refined and unrefined sugar, this food enzyme is added to sliced sugar beet during extraction to obtain raw juices.[36] The raw juices are subject to downstream processing, such as purification, evaporation and crystallisation, to obtain refined sucrose with molasses as a by-product. The food enzyme–TOS is removed from the refined sucrose, but remains in molasses (EFSA CEP Panel, 2021).

During coffee bean demucilation, the food enzyme is added to the harvested coffee cherry.[38] The hydrolysis of mucilage by pectin lyases facilitates the separation of the coat from the green coffee beans. The food enzyme–TOS is removed from the demucilated coffee beans by repeated washing (EFSA CEP Panel, 2021).

Based on data provided on thermostability (see Section 3.3.1), the Panel expected that the food enzyme is inactivated in final foods by heat during all the food manufacturing processes except in juices and wine.

[33] Technical dossier/volume I/p.53.
[34] Additional information September 2021/Response to question 10.
[35] Technical dossier/volume I/p.52.
[36] Additional information September 2021.
[37] Technical dossier/volume I/p.54.
[38] Technical dossier/volume I/p.55.

3.5.2. Dietary exposure estimation

In accordance with the guidance document (EFSA CEP Panel, 2021), 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 juices production and products other than juices (purees only); wine and wine vinegar production; refined and unrefined sugar production.

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, 2021). 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 41 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). The highest dietary exposure was estimated to be about 0.2 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)	0.002–0.021 (11)	0.007–0.076 (15)	0.009–0.052 (19)	0.003–0.028 (21)	0.002–0.017 (22)	0.002–0.012 (22)
Min–max 95th (number of surveys)	0.006–0.077 (9)	0.035–0.122 (13)	0.033–0.200 (19)	0.012–0.138 (20)	0.010–0.068 (22)	0.008–0.044 (21)

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	+
Not only purees were included in the calculation, but also other fruit and vegetable products (e.g. canned fruits, jam)	+

Sources of uncertainties	Direction of impact
Minor FoodEx categories found to only sporadically contain molasses were excluded from the exposure assessment	–
'Brown sugar' produced through use of cane molasses or caramelised sugar syrup was excluded, due to it being a niche product on the European market	–
The transfer of food enzyme–TOS into cane and beet molasses/syrups was assumed to be 100%	+
No distinction was made between beet molasses and cane syrups used as ingredients in foods	+/–
Use of recipe fractions in disaggregation FoodEx categories	+/–
Use of technical factors in the exposure model	+/–
Exclusion of other processes from the exposure assessment – Fruit and vegetable processing for fruit brandies – Coffee bean demucilation	–

+: 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 overestimation of the exposure.

The exclusion of two food manufacturing process (fruit and vegetable processing for fruit brandies, coffee bean demucilation) from the exposure assessment was based on > 99% of TOS removal during these processes and was not expected to have an impact on the overall estimate derived.

3.6. Margin of exposure

The comparison of the NOAEL (1,000 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0.002–0.076 mg TOS/kg bw per day at the mean and from 0.006 to 0.200 mg TOS/kg bw per day at the 95th percentile resulted in margin of exposure (MoE) of at least 5,000.

4. Conclusions

Based on the data provided and the derived margin of exposure, the Panel concluded that the food enzyme pectin lyase produced with the genetically modified *Trichoderma reesei* strain RF6199 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.

Documentation provided to EFSA

1) Dossier "Application for authorisation of a pectin lyase from a genetically modified strain of *Trichoderma reesei* in accordance with Regulation (EC) No 1331/2008", March 2014. Submitted by AB Enzymes GmbH.
2) Summary report on technical data and dietary exposure related to pectin lyase from a genetically modified strain of *Trichoderma reseei* (strain RF6199) by AB Enzymes. Delivered by Hylobates Consulting and BiCT (Rome, Italy) on 16 February 2015.
3) Summary report on genotoxicity, subchronic toxicity study and allergenicity related to pectin lyase from *Aspergillus niger* produced with *Trichoderma reesei* (strain RF6199) by AB Enzymes. Delivered by FoBiG (Freiburg, Germany) on 27 June 2014.
4) Additional information. September 2021. Submitted by AB Enzymes GmbH.
5) Additional information June 2022. Submitted by AB Enzymes GmbH.

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Abbreviations

AMFEP	Association of manufacturers and formulators of enzyme product
CAS	Chemical Abstracts Service
CBS	Westerdijk Fungal Biodiversity Institute
CEF	EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
FAO	Food and Agriculture Organisation of the United Nations
GMO	genetically modified organisms
IUBMB	International Union of Biochemistry and Molecular Biology
ITS	internal transcribed spacers

PCR	polymerase chain reaction
PTF	Pectintranselimanse units
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TOS	total organic solids
WGS	Whole genome sequencing
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 https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2022.7675#support-information-section).

The file contains two sheets, corresponding to two tables.

Table 1: Mean 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
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).