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Safety evaluation of the food enzyme endo-polygalacturonase from the genetically modified *Trichoderma reesei* strain RF6197

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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 *Trichoderma reesei* strain RF6197 by AB Enzymes GmbH. The genetic modifications do not give rise to safety concerns. The food enzyme was considered 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 juices, production of wine and wine vinegar, coffee demucilation and production of plant extracts as flavouring preparations. Since residual amounts of the total organic solids (TOS) are removed during the coffee demucilation and the production of flavouring extracts, dietary exposure was calculated only for the remaining three food processes. It was estimated to be up to 0.156 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, resulted in a margin of exposure of at least 6,410. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and matches were found with a number of pollen allergens. The Panel considered that, under the intended conditions of use, the risk of allergic reactions by dietary exposure, particularly in individuals sensitised to pollen 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, pectinase, *Trichoderma reesei*, 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 EU market and intended to remain on that market, as well as all new food enzymes, shall be subjected to a safety evaluation by the European Food Safety Authority (EFSA) and approval via an EU Community list.

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

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

1.1.1. Background as provided by the European Commission

Only food enzymes included in the 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.

Four applications have been submitted by the companies 'Novozymes A/S' and 'AB Enzymes GmbH' for the authorisation of the food enzymes Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-AV), Beta-glucanase, Xylanase and Cellulase produced by a strain of *Humicola insolens* (strain NZYM-ST), Polygalacturonase from a genetically modified strain of *Trichoderma reesei* (strain RF6197) and Pectin esterase from a genetically modified strain of *Trichoderma reesei* (strain RF6201).

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 four applications fall within the scope of the food enzyme Regulation and contain all the elements required under Chapter II of that Regulation.

[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, p. 15–24.

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 Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-AV); Beta-glucanase, Xylanase and Cellulase produced by a strain of *Humicola insolens* (strain NZYM-ST); Polygalacturonase from a genetically modified strain of *Trichoderma reesei* (strain RF6197) and Pectin esterase from a genetically modified strain of *Trichoderma reesei* (strain RF6201) 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 endo-polygalacturonase from the genetically modified *Trichoderma reesei* strain RF6197.

2. Data and Methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme endo-polygalacturonase from a genetically modified *T. reesei* (strain RF6197).

Additional information was requested from the applicant during the assessment process on 25 February 2022 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) and following the relevant guidance documents of the EFSA Scientific Committee.

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 juices, production of wine and wine vinegar, coffee demucilation and the production of plant extracts as flavouring preparations.

3.1. Source of the food enzyme

The endo-polygalacturonase is produced with the genetically modified filamentous fungus *T. reesei* strain RF6197, which is deposited in the Westerdijk Fungal Biodiversity Institute culture collection (CBS, the Netherlands) with the deposit number [redacted].[4] The production strain was identified as

[4] Technical dossier/Volume III/Appendix 11.

T. reesei [redacted].[5]

3.1.1. Characteristics of the parental and recipient microorganisms

The parental strain was [redacted].[6] [redacted].[7]

3.1.2. Characteristics of introduced sequences

The sequence encoding the endo-polygalacturonase [redacted].[8]

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].[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* RF6197 differs from the recipient strain in its capacity to produce the endo-polygalacturonase [redacted].[11]

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

[5] Technical Dossier/Additional information July 2022/Annex 1.
[6] Technical Dossier/Volume III/GM part and Appendix 2.
[7] Technical Dossier/Volume III/Appendix 1B.
[8] Technical Dossier/Volume III/GM part and Appendices 7 and 8.
[9] Technical Dossier/Volume III/GM part and Appendix 9.
[10] Technical Dossier/Volume III/Appendix 10.
[11] Technical dossier/Volume III/Appendix 12.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[12], with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[13]

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. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in which the enzyme protein is retained, while most of the low molecular mass material passes the filtration membrane and is discarded.[14] The applicant provided information on the identity of the substances used to control the fermentation and in the subsequent downstream processing of the food enzyme.[15]

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.[16] The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be [redacted] kDa.[17] The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). A consistent protein pattern was observed across all batches. The gel showed a major protein band of about [redacted] kDa, corresponding to a glycosylated form of the enzyme. The food enzyme was tested for β-glucanase, cellulase and xylanase activities and all were detected.[18] 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: pH 3.9, 30°C, 11 min). The enzymatic activity is expressed in polygalacturonase units (PGU/mg) and calculated based on the comparison to the enzymatic activity value of a known standard sample.[19]

The food enzyme has a temperature optimum around 50°C (pH 3.9) and a pH optimum around pH 4.5 (40°C). Thermostability was tested after a pre-incubation of the food enzyme at 85°C for different periods (pH 4.5). The endo-polygalacturonase activity decreased by more than 80% after 1 min of pre-incubation and was not detected after 7 min.[20]

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).[21] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation was 25.1% and the mean enzyme activity/TOS ratio was 5,158,197 PGU/mg TOS.

[12] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of food additives. OJ L 226, 25.6.2004, pp. 3–21.
[13] Technical Dossier/Volume I/pp. 41–42 and Volume II/Annex 9.
[14] Technical Dossier/Volume I/pp. 41–50 and Volume II/Annexes: 10, 13.
[15] Technical Dossier/Volume I/pp. 42–43 and Volume II/Annexes: 11, 12, 14.
[16] Technical Dossier/Volume I/pp. 32 and Volume II/Annex 4.
[17] Technical Dossier/Additional information July 2022/RF6197/Additional information July 2022/Annex 5.
[18] Technical Dossier/Volume I/pp. 31, 34.
[19] Technical Dossier/Volume I/pp. 33 and Volume II/Annex 3.
[20] Technical Dossier/Volume I/pp. 34–35 and Volume II/Annexes: 3, 5.
[21] Technical Dossier/Volume I/pp. 30, 67-68/Volume II/Annexes: 1, 2, 16/Additional information July 2022/Annex 6.

Table 1: Composition of the food enzyme

Parameters	Unit	Batches			
		1	2	3	4[(a)]
Endo-polygalacturonase activity	PGU/mg[(b)]	1,220,000	1,170,000	1,500,000	6,680,000
Protein	%	20.6	19.9	23.4	75.5
Ash	%	0.3	0.2	0.3	0.9
Water	%	75.5	76.3	72.2	6.7
Total organic solids (TOS)[(c)]	%	24.2	23.5	27.5	92.4
Activity/TOS	PGU/mg TOS	5,041,322	4,978,723	5,454,545	7,229,000

(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 commercial batches and in the batch used for toxicological studies was below 5 mg/kg,[22] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[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).[22] No antimicrobial activity was detected in any of the tested batches.[22]

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 T2-toxin and HT2-toxin was examined in the four food enzyme batches and all were below the limit of quantification (LoQ) of the applied methods.[22,24] 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 [redacted].[25] No colonies of the production strain were detected. A positive control was included.

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

3.4. Toxicological data

A battery of toxicological tests, including a bacterial reverse mutation test (Ames test), an *in vitro* mammalian chromosomal aberration test and a repeated dose 90-day oral toxicity study in rats, was provided. Although having a slightly higher activity/TOS value as compared to those of the commercial batches, batch 4 (Table 1) was considered suitable as a test item.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

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

[22] Technical Dossier/Volume I/pp. 31-31/Volume II/Annexes: 1, 2, 16/Additional information July 2022/Annex 6.
[23] LoQ: Pb = 0.05 mg/kg.
[24] LoQs: T2 and HT2-toxin = 10 μg/kg each.
[25] Technical Dossier/Additional information July 2022/Annexes 2 and 3.
[26] Technical Dossier/Additional information July 2022/Annex 4.
[27] Technical dossier/Annex 17.

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 (experiment I) and the preincubation method (experiment II). Two separate experiments in triplicate were carried out using eight concentrations of the food enzyme in the first experiment (3, 10, 33, 100, 333, 1,000, 2,500 and 5,000 μg TOS/plate), and six concentrations of the food enzyme in the second experiment (33, 100, 333, 1,000, 2,500 and 5,000 μg TOS/plate).

No cytotoxicity was observed at any concentration tested. In the first experiment *S. Typhimurium* strain TA1537 showed an increase in revertant colony numbers at 5,000 μg TOS/plate (2.4-fold) in the absence of S9-mix. However, this was not reproduced in the second experiment (1.6-fold increase). In the presence of S9-mix, *S. Typhimurium* TA1537 at 5,000 μg TOS/plate showed an increase in revertant colony numbers in both experiments (2.1-fold and 2.5-fold). However, the reported values were within the ranges of the background values for these strains and were considered not biologically relevant. Upon treatment with the food enzyme, there was no significant increase in revertant colony numbers above the control values in the other strains with or without S9-mix.

The Panel concluded that the food enzyme endo-polygalacturonase did not induce gene mutations under the test conditions applied 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]

A dose-finding study was performed at concentrations of food enzyme ranging from 676.4 to 5,411 μg/mL, and no inhibition of cell growth by ≥ 50% was observed. Based on these results, in the first experiment the cells were exposed to the food enzyme at 3,607.3, 4,509.2 and 5,411 μg/mL (corresponding to 3,334, 4,167 and 5,000 μg TOS/mL) in the short-term treatment (4 h followed by 14 h recovery period) with and without metabolic activation (S9-mix). In the second experiment, a continuous treatment (18 h) in the absence of S9-mix was performed at the same concentrations of the food enzyme. No cytotoxicity was observed at any concentration level of the test substance.

The frequency of structural chromosomal aberrations was statistically significantly increased at 3,607.3 and 4,509.2 μg/mL (corresponding to 3,334 and 4,167 μg TOS/mL) in the continuous treatment (18 h), in the absence of S9-mix, in the second experiment (3.5% and 3.0% vs. 0.5% in the control). This increase was not concentration-dependent and the values were within the range of the laboratory historical control data (0–4%). Therefore, it was not considered biologically relevant.

The Panel concluded that the food enzyme endo-polygalacturonase did not induce structural and numerical chromosomal aberrations under the test conditions employed for this study.

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

The repeated dose 90-day oral toxicity study was performed in accordance with OECD Test Guideline 408 (OECD, 1998) and following GLP.[29] Groups of 10 male and 10 female RccHanTM: WIST (SPF) rats received by gavage the food enzyme in amounts corresponding to 100, 300 and 1,000 mg TOS/kg bw per day. Controls received the vehicle (bidistilled water).

No mortality was observed.

A statistically significant increase in the mean body weight gain, compared to controls, was observed in mid-dose females on day 43 of treatment (+19%). The Panel considered the change as not toxicologically relevant as it was only recorded sporadically, in one sex, there was no dose–response relationship and the change was without a statistically significant effect on the bw throughout the dosing period and the final bw gain.

The functional observations revealed a statistically significantly increased locomotor activity in high-dose males during 0–10 min (+18%), 10–20 min (+115%) and for an overall increase from 0–60 min (+44%). In mid-dose males, an increased locomotor activity was observed during several intervals (0–10, 10–20, 20–30 and 30–40 min) (+18%, +94%, +163%, +246%, respectively), and for an overall increase from 0–60 min (+63%). In high-dose females, a statistically significant increase in locomotor activity was recorded during 0–10 min only (+53%). The Panel considered the changes as not toxicologically relevant as there was no consistency between changes in males and females, there was no dose–response relationship (for mid-dose males in the intervals 20–30 min and 30–40 min and for

[28] Technical dossier/Annex 18.
[29] Technical dossier/Annex 19.

mid- and high-dose males in overall increase), and the change in high-dose females was only recorded sporadically.

Haematological investigation revealed a statistically significant increase in haematocrit (HCT) (+5%) and basophil counts (+50%) in high-dose males. In mid- and high-dose males, lower methaemoglobin (MET-HB) levels were reported (−27%, −18%, respectively). In females, a statistically significantly lower haemoglobin (HB) levels in mid- and high-dose groups (−4.3%, −3.2%, respectively), a lower methaemoglobin level (−18%) and a higher prothrombin time (PT) (+6%) in the high-dose group were reported. The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (except lower MET-HB levels), the magnitude of the change was low (HCT, basophils, PT), there was no dose–response relationship (MET-HB in males, HB in females) and the changes were within the historical control values.

Clinical chemistry investigation revealed a statistically significant increase in sodium in high-dose males (+0.9%) and females (+2.2%). In high-dose females, higher chloride level (+4.2%) and lower triglyceride level (−32%) were reported. The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (chloride, triglycerides), the changes were within the historical control values (except sodium in high-dose females that was slightly higher than laboratory historical control value) and they were recorded in the absence of histopathological changes in the kidneys (sodium).

Statistically significant changes in organ weights in males included an increase in absolute kidney weight (+12.5%), kidney-to-brain weight ratio (+14%) and in liver-to-brain weight ratio (+19%) in high-dose group, a decrease in absolute heart weight (−7.3%) in low-dose group, and a decrease in heart-to-body weight (−8%, −8%, respectively) in low- and mid-dose groups. Statistically significant changes in organ weights in females included an increase in absolute uterus/oviduct weight (+62%) and the relative uterus/oviduct-to-body weight (+71%) and uterus/oviduct-to-brain weight (+63%) in the high-dose group. The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (kidney, liver heart), there was no dose–response relationship (heart), the changes were small (heart, kidney), there was no change in the absolute and the relative to bw (liver) or in the relative weight (kidney), there were no changes in clinical-chemistry parameters indicative of kidney and liver function, there were no changes in urinalysis and there were no histopathological changes in any of the organs. The observed increase in uterus weight and in uterus relative weights was attributed to a single animal with a cyst on the uterus horn.

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.

3.4.2. Allergenicity

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

The potential allergenicity of the endo-polygalacturonase produced with the genetically modified *T. reesei* strain RF6197 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, several matches were found. The matching allergens were pollen allergens from maize (*Zea mays*), bahia grass (*Paspalum notatum*), Japanese cedar (*Cryptomeria japonica*), Mountain cedar (*Juniperus ashei*), common timothy-grass (*Phleum pratens)*, trumpet lily (*Lilium longiflorum*) and London plane tree (*Platanus acerifolia*), all known as respiratory allergens.[30]

No information is 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).

[30] Technical Dossier/Volume I/pp. 69–72 and Volume II/Annex 20/Additional information July 2022/Annex 9.

[redacted], products that may cause allergies or intolerances (Regulation (EU) No 1169/2011[31]), are used as raw materials. 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 of these materials 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 manufacturing 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[32]

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	**3**
Fruit and vegetable processing for products other than juices	Fruits and vegetables	**6**
Production of wine and wine vinegar	Grapes	**2**
Coffee demucilation	Coffee cherries	0.5
Production of plant extracts as flavouring preparations[33]	Fruit and vegetables	300

TOS: total organic solids.
(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.

In fruit and vegetable processing, 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 or vegetables and to the pressed juice before clarification and filtration.[34] 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. 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. The food enzyme–TOS remains in these products.

In wine and wine vinegar production, the endo-polygalacturonase is often added together with other cell wall hydrolytic enzymes during crushing. It can be added also during the maceration and clarification steps.[36] Such enzymatic treatment aids pressing and facilitates the extraction of aromatic compounds. The food enzyme–TOS may remain in wine and wine vinegar.

[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/p. 59 and Additional information July 2022/Answers 11 and 12.
[33] Additional data July 2022/Answer 13.
[34] Technical dossier/p. 54.
[35] Technical dossier/p. 55.
[36] Technical dossier/p. 56.

In coffee bean demucilation, the endo-polygalacturonase is added to green coffee cherries during pulping and fermentation to degrade the mucilage.[37] The food enzyme–TOS is removed during the subsequent washing steps (EFSA CEP Panel, 2021b).

The food enzyme is used to obtain aroma concentrates or essential oils for use as flavouring preparations. 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.[33] The aroma concentrates are primarily used in the reconstitution of juices.

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

Based on data provided on thermostability (see Section 3.3.1), the endo-polygalacturonase is expected to be inactivated by heat in most of the food processes, but may remain active in wine and wine vinegar as well as in juices, depending on the pasteurisation conditions.

3.5.2. Dietary exposure estimation

In accordance with the guidance document (EFSA CEP Panel, 2021a), dietary exposure was calculated only for food manufacturing processes where the food enzyme–TOS remains in the final foods: fruit and vegetable processing for juice production, fruit and vegetable processing for products other than juice and 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 bw. This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only 1 day per subject were excluded and high-level exposure/intake was calculated for only those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011).

Table 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 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). The highest dietary exposure to the food enzyme–TOS was estimated to be 0.156 mg TOS/kg bw per day in infants at the 95th percentile.

Table 3: Summary of the 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)	0.005–0.081 (12)	0.016–0.096 (15)	0.009–0.063 (19)	0.003–0.033 (21)	0.004–0.021 (22)	0.002–0.020 (23)
Min–max 95th percentile (number of surveys)	0.018–0.156 (11)	0.064–0.147 (14)	0.033–0.133 (19)	0.013–0.089 (20)	0.014–0.067 (22)	0.011–0.046 (22)

TOS: total organic solids.

[37] Technical dossier/p. 57.
[38] Additional data July 2022/Annex 10.
[39] Additional data July 2022/Annex 11.

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 – Production of plant extract as flavouring preparations – Coffee demucilation	–

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

The conservative approach applied to estimate the dietary 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 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 0.002–0.096 mg TOS/kg bw per day at the mean and from 0.011–0.156 mg TOS/kg bw per day at the 95th percentile resulted in a margin of exposure (MoE) of at least 6,410.

4. Conclusions

Based on the data provided, the removal of TOS during coffee demucilation and the production of flavouring extracts, 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 *T. reesei* strain RF6197 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

1) Dossier "Application for authorisation of a polygalacturonase from a genetically modified strain of *Trichoderma reesei* in accordance with Regulation (EC) No 1331/2008". October 2014. Submitted by AB Enzymes GmbH.
2) Summary report on the GMM part for polygalacturonase produced by *Trichoderma reseei* strain RF6197 by AB Enzymes. Delivered by Pedersen and Eriksen (Kongens Lyngby, Denmark) on 3 February 2016.

3) Summary report on technical data and dietary exposure related to polygalacturonase from a strain of *Trichoderma reesei* (strain RF6197) by AB Enzymes. Delivered by Hylobates Consulting and BiCT (Rome, Italy) on 23 February 2016.
4) Additional information. July 2022. Submitted by AB Enzymes GmbH.

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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
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
LoQ	limit of quantification
MoE	margin of exposure
OECD	Organisation for Economic Cooperation and Development
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
TOS	total organic solids
WHO	World Health Organization

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

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

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

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

Appendix B – Population groups considered for the exposure assessment

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From 12 weeks on up to and including 11 months of age	Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia, Spain
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Netherlands, Portugal, 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).