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Safety evaluation of the food enzyme pectin lyase from the genetically modified *Aspergillus luchuensis* strain FLOSC

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), Claude Lambré, José Manuel Barat Baviera, Claudia Bolognesi, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis, Holger Zorn, Boet Glandorf, Lieve Herman, Magdalena Andryszkiewicz, Davide Arcella, Natália Kovalkovičová, Yi Liu and Andrew Chesson

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 *Aspergillus luchuensis* (formally *Aspergillus niger*) strain FLOSC by Advanced Enzyme Technologies Ltd. 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. The food enzyme is intended to be used in fruit and vegetable processing for juice production. Based on the maximum use level and individual data from the EFSA Comprehensive European Food Database, dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.268 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 794 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 2,900. A search for 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 sensitisation and elicitation reactions by dietary exposure cannot be excluded, but the likelihood for this to occur is considered to be 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, *Aspergillus luchuensis*, genetically modified microorganism

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Correspondence: fip@efsa.europa.eu

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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[1] 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 CEF Panel, 2009) 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[1] on food enzymes.

Five applications were introduced by the companies 'Novozymes A/S', 'DSM Food Specialties B.V', 'Advanced Enzyme Technologies LtD' and 'the Association of Manufacturers and Formulators of Enzyme Products (AMFEP)' for the authorization of the food enzymes pullulanase from a genetically modified strain of *Bacillus subtilis* (strain NZYM-AK), glucoamylase from a genetically modified strain of *Aspergillus niger* (strain NZYM-BW), chymosin from a genetically modified strain of *Kluyveromyces lactis* (strain CHY), pectin lyase from a genetically modified strain of *Aspergillus niger* (strain FLOSC) and triacylglycerol lipase from pregastric tissues of cattle, goat and sheep, respectively.

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008[2], the Commission has verified that the five 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, 11.3.2011, pp. 15–24.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments of the food enzymes pullulanase from a genetically modified strain of *Bacillus subtilis* (strain NZYM-AK), glucoamylase from a genetically modified strain of *Aspergillus niger* (strain NZYM-BW), chymosin from a genetically modified strain of *Kluyveromyces lactis* (strain CHY), pectin lyase from a genetically modified strain of *Aspergillus niger* (strain FLOSC) and triacylglycerol lipase from pregastric tissues of cattle, goat and sheep in accordance with Article 17.3 of Regulation (EC) No 1332/2008[1] 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 the genetically modified *Aspergillus luchuensis* strain FLOSC, initially identified as *Aspergillus niger*.

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 a genetically modified *A. niger* agg. (strain FLOSC).

Additional information was requested from the applicant during the assessment process on 24 March 2021 and received on 7 June 2021 (see 'Documentation provided to EFSA').

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA 'Guidance on transparency in the scientific aspects of risk assessment' (EFSA, 2009) and following the relevant guidance documents of EFSA Scientific Committee.

The current Guidance on the submission of a dossier on food enzymes for safety evaluation (EFSA CEF Panel, 2009) 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 to the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021a).

3. Assessment[4]

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 lyase catalyses a β-eliminitive 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.[5] The enzyme is intended to be used in fruit and vegetable processing for juice production.[6]

3.1. Source of the food enzyme[6]

The pectin lyase is produced with the genetically modified filamentous fungus *A. luchuensis* strain FLOSC (formerly *A. acidus*), which is deposited at [redacted] with deposit number [redacted][7]

[4] Technical dossier/2 Summary/p. 4–5; Technical dossier/3.2 Risk assessment data/p. 3.
[5] Technical dossier/2 Summary/p. 5, 8; Technical dossier/3.2 Risk assessment data/p. 9, 36.
[6] Technical dossier/Additional information, 7 June 2021.
[7] Technical dossier/Additional information, 7 June 2021/Annex 1.

The production strain was identified as *A. luchuensis* [redacted][8]

3.1.1. Characteristics of the parental and recipient microorganisms[9]

The parental strain is [redacted].[6] The recipient strain [redacted]

3.1.2. Characteristics of introduced sequences

[redacted]

3.1.3. Description of the genetic modification process

[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 *A. luchuensis* FLOSC differs from the recipient strain in its capacity to produce the pectin lyase [redacted] The absence of the antibiotic resistance genes used during the genetic modification was confirmed by PCR analysis.

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

3.2. Production of the food enzyme[11]

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 [redacted] with conventional process controls in place. After completion of the fermentation, [redacted]

[8] Technical dossier/Annex I1.
[9] Technical dossier/Annex I2; Technical dossier/3.2 Risk assessment data/p. 19–21; Technical dossier/Additional information, 7 June 2021.
[10] Technical dossier/Additional information, 7 June 2021/Annexure A.
[11] Technical dossier/3.2 Risk assessment data/p. 30–35; Technical dossier/Annex F and Annex G/Appendix 2.
[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/3.2 Risk assessment data/p. 30; Technical dossier/Annex F.

[redacted] Finally, the food enzyme is [redacted][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 considers 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[6]

The pectin lyase is a single polypeptide chain of 379 amino acids.[16] The molecular mass of the mature protein, calculated from the amino acid sequence, is 39.8 kDa.[6] The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis that showed a major protein band corresponding to an apparent molecular mass consistent with the calculated molecular mass of the enzyme,[17] plus several minor bands. A consistent protein pattern was observed across all batches. No other enzymatic activities were reported.[18]

The in-house determination of enzyme activity is based on the cleavage of citrus pectin (reaction conditions: pH 5.5, 45°C, 10 min). The enzymatic activity is determined by measuring the release of unsaturated Δ-4,5 polygalacturonides, which are determined spectrophotometrically at 235 nm. Enzyme activity is expressed in Pectin Lyase Units/g (PLU/g). One unit of pectin lyase (PLU) is defined as the quantity of the enzyme forming one micromole of unsaturated Δ-4,5 uronide in one minute under the standard assay conditions.[19]

The food enzyme has a temperature optimum around 50°C (pH 5.5) and a pH optimum around pH 5.5 (45°C). Thermostability was tested after a pre-incubation of the food enzyme for 120 min at different temperatures (pH 5.5). Enzyme activity decreased above 55°C showing no residual activity above 70°C.[20]

3.3.2. Chemical parameters[21]

Data on the chemical parameters of the food enzyme were provided for three batches of a dried preparation, one of which (batch 3) was used for the toxicological tests (Table 1).[22] The average total organic solids (TOS) of the three food enzyme batches is 79.2% and the average enzyme activity/mg TOS ratio is 11.9 PLU/mg TOS. Prior to drying the food enzyme is stabilized with [redacted][23]

Table 1: Composition of the food enzyme[6]

Parameters	Unit	Batch		
		1	2	3[(a)]
Pectin lyase activity	PLU/g batch[(b)]	9,565	9,045	9,864
Protein	%	44.6	42.3	46.7
Ash	%	8.26	9.24	7.25
Water	%	6.3	7.1	6.18
[redacted]	%	4.34	6.66	6.25
Total organic solids (TOS)[(c)]	%	81.1	77.0	80.32
Pectin lyase activity/mg TOS	PLU/mg TOS	11.80	11.75	12.28

(a): Batch used for the toxicological studies.
(b): PLU: Pectin Lyase Units (see Section 3.3.1).
(c): TOS calculated as 100% – % water – % ash – % [redacted]

[14] Technical dossier/3.2. Risk Assessment Data/p. 30–35; Technical dossier/Annex G.
[15] Technical dossier/Annex G/Appendix 2.
[16] Technical dossier/2 Summary/p. 5; Technical dossier/3.2 Risk assessment data/p. 5.
[17] Technical dossier/Annex B; Technical dossier/3.2 Risk assessment data/p. 4; Technical dossier/Additional information, 7 June 2021.
[18] Technical dossier/3.2. Risk Assessment Data/p. 10.
[19] Technical dossier/Annex A2., Annex C.
[20] Technical dossier/Annex C; Technical dossier/3.2 Risk assessment data/p. 11–12.
[21] Technical dossier/3.2. Risk Assessment Data/p. 4, 6, 33, 50; Technical dossier/Annex A3; Annex J; Technical dossier/Additional information, 7 June 2021.
[22] Technical dossier/3.2. Risk Assessment Data/p. 4, 6, 50; Technical dossier/Annex A3; Annex J.
[23] Technical dossier/Additional information, 7 June 2021; Technical dossier/Annex G.

3.3.3. Purity[24]

The lead content in the three commercial batches was below 0.25 mg/kg[25] which complies with the specification for lead (≤ 5 mg/kg) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006). In addition, the levels of arsenic, mercury and cadmium were below the limits of detection (LODs) of the employed methodologies.[26,27]

The food enzyme complies with the microbiological criteria (for total coliforms, *Escherichia coli* and *Salmonella*) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006).[28] No antimicrobial activity was detected in any of the tested batches.[29]

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 and M1, ochratoxin A, fumonisin B1, zearalenone, deoxynivalenol, T-2 toxin, HT2-toxin, ergocornine, ergocristine, ergocryptine, ergometrine, ergosine, ergotamine was examined in the three food enzyme batches. All were below the LOD of the applied methods.[30,31] Adverse effects derived from 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[32]

The absence of viable cells of the production strain in the food enzyme was demonstrated in three independent batches analysed in triplicate.[33] [redacted] No colonies were produced.

The absence of recombinant DNA in the food enzyme was demonstrated by PCR analysis of three batches in triplicate. No DNA was detected with [redacted] with a limit of detection of 10 ng spiked DNA/g food enzyme.[34]

3.4. Toxicological data[35]

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 3 (Table 1) which was produced according to the procedure used for commercial batches and is considered suitable as a test item.

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).[36] Five strains of *Salmonella* Typhimurium (TA100, TA102, TA97a, TA98 and TA1535) were used in the presence or absence of metabolic activation (S9-mix), applying the standard plate incorporation method. Two separate experiments were carried out in triplicate, using five different concentrations of the food enzyme, ranging from 61.7 to 5,000 μg/plate, corresponding to

[24] Technical dossier/2 Summary/p. 4; Technical dossier/Annex A1; Annex A3/Appendix 1; Annex D; Annex E1; Annex I1; Technical dossier/3.2 Risk assessment data/p. 6, 9; Technical dossier/Additional information, 7 June 2021.
[25] Technical dossier/3.2. Risk Assessment Data/p. 9; Technical dossier/Annex A1; Annex D.
[26] Technical dossier/Annex D.
[27] LODs: Pb, As, Cd = 0.25 mg/kg; Hg = 0.025 mg/kg.
[28] Technical dossier/3.2. Risk Assessment Data/p. 6, 9; Technical dossier/Annex A1; Annex A3.
[29] Technical dossier/3.2 Risk assessment data/p. 6, 9; Technical dossier/Annex A1; Annex A3, Annex E2.
[30] Technical dossier/3.2 Risk assessment data/p. 6, 9; Technical dossier/Annex A1, Annex E1; Annex I1.
[31] Technical dossier/Annex I1; Technical dossier/Additional information, 7 June 2021: LODs: aflatoxins (B1, B2, G1, G2, M1) = 1 μg/kg each; fumonisin B1 = 0.50 μg/kg; ochratoxin A = 1 μg/kg; T-2 toxin = 10 μg/kg; HT2-toxin = 50 μg/kg; zearalenone = 5 μg/kg; deoxynivalenol = 100 μg/kg; ergocornine, ergocristine, ergocryptine, ergometrine, ergosine, ergotamine = 100 μg/kg each.
[32] Technical dossier/Additional information, 7 June 2021/Annexure B and Annexure C.
[33] Technical dossier/Annex M/Appendix 1.3.
[34] Technical dossier/Annex M; Technical dossier/Additional information, 7 June 2021/Annexure B and Annexure C.
[35] Technical dossier/3.2 Risk assessment data/p. 43–52; Technical dossier/Annex J.
[36] Technical dossier/Annex J.

49, 147, 441, 1,323 and 3,970 μg TOS/plate. No cytotoxicity was observed at any concentration of the test substance. Upon treatment with the food enzyme there was no biologically relevant increase in the number of revertant colonies above the control values in any strain tested, with or without S9-mix.

The Panel concluded that the food enzyme pectin lyase 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 according to OECD Test Guideline 473 (OECD, 2014) and following GLP.[37] An experiment was performed with duplicate cultures of human peripheral blood lymphocytes. The cell cultures were treated with the food enzyme either with or without metabolic activation (S9-mix). The range-finding study was performed at concentrations ranging from 312 to 5,000 μg/mL, and no inhibition of mitotic activity above 50% was seen. Based on these results, cells were exposed to the food enzyme and scored for chromosomal aberrations at concentrations of 1,250, 2,500 and 5,000 μg/mL, corresponding to 993, 1,985 and 3,970 μg TOS/mL, in a short-term treatment (4 h exposure and 20 h recovery period) either with and without S9-mix, and in the continuous treatment (24 h) in the absence of S9-mix. No cytotoxicity was seen either in the short-term (with or without S9-mix) or in the long-term treatment. The frequency of structural and numerical aberrations was not statistically significantly different to the negative controls at any concentration tested.

The Panel concluded that food enzyme pectin lyase did not induce increase in the frequency of structural and numerical aberrations under the test conditions applied in 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 OECD Test Guideline 408 (OECD, 1998) and following GLP.[38] Groups of 10 male and 10 female Sprague-Dawley rats received by gavage the food enzyme in doses of 250, 500 and 1,000 mg/kg bw per day, corresponding to 198.5, 396 and 794 mg TOS/kg bw per day. Controls received the vehicle (distilled water).

Furthermore, a recovery control and a high-dose groups were included in the study, each comprising 6 males and 6 females, and terminated 4 weeks after the end of treatment.

No mortality was observed.

In the functional observations, a statistically significant increase in motor activity (+42.5%) in mid-dose males and a statistically significant decrease (−48.2%) in male recovery group for the first interval were observed. In the female recovery group, a statistically significant increase in motor activity was observed for the first and second interval (+87.4% and +55.9%, respectively). The Panel considered these changes as incidental.

Haematological investigation revealed a statistically significant decrease in mean corpuscular haemoglobin concentration (MCHC) (−1%) and platelets (−20%) in low-dose males. A statistically significant increase in mean corpuscular volume (MCV) in low-, mid- and high-dose females (+4%, +2%, +4%), and a statistically significant decrease in total red blood cells (RBC, −11%) in high-dose females were reported. At the end of the recovery period, a statistically significant decrease in platelets (−15%) in the male recovery group was noted. The Panel considered all these changes as not toxicologically relevant as they were only observed in one sex (all parameters), in absence of a dose–response relationship (MCHC, platelets, MCV), because the changes were low (MCHC, platelets, MCV) and the change was only observed at the end of recovery (platelets).

Clinical chemistry investigation revealed a statistically significant increase in phosphorus in low-, mid-and high-dose males (+13%, +14%, +10%), an increase in sodium in mid- and high-dose males (+5%, +4%), an increase in total protein level (+9%), alanine aminotransferase (ALT, +18%) and globulin (+9%) in mid-dose males. In addition, a statistically significant decrease in calcium in mid- and high-dose males (−9%, −5%), a decrease in gamma glutamyl transferase (GGT, −19%) and bilirubin (−38%) in high-dose males was observed. In females, a statistically significant increase in aspartate aminotransferase (AST, +22%) in high-dose group, an increase in total protein level (+7%), calcium (+4%) and globulin (+7%) in mid-dose group, and phosphorus (+18%) in low-dose group was noted. In addition, a statistically significant decrease in chloride in low- and mid-dose females

[37] Technical dossier/Annex J; Technical dossier/Additional information, 7 June 2021/Annex 3.
[38] Technical dossier/Annex J/p. 114–647; Technical dossier/Additional information, 7 June 2021/Annex 3.

(−3%, −7%) was observed. At the end of the recovery period, a statistically significant increase in sodium (+2.5%) in male recovery group was observed. A statistically significant decrease in chloride (−1.7%) in male recovery group and calcium (−5.3%) in female recovery group were reported.

The Panel considered all these changes as not toxicologically relevant as they were only observed in one sex (ALT, AST, GGT, bilirubin, sodium), in the absence of a dose-response relationship (total protein, ALT, globulin, calcium, phosphorus, sodium, chloride) and because of a low magnitude of the changes (ALT, AST, GGT, total proteins, globulin, calcium, sodium, chloride).

Statistically significant changes in organ weights included an increase in relative liver weight in low- and mid-dose females (+12%, +13%), relative thymus weight in low-, mid- and high-dose females (+46%, +29%, +39%), and a decrease in relative weights of ovaries (−17%) and uterus (−20%) in high-dose female group. At the end of the recovery period, there was a statistically significant increase in relative spleen weight (+14%) in male recovery group and in relative weight of ovaries (+29%) and heart (+27%) in female recovery group. In addition, in female recovery group a statistically significant decrease in relative liver (−18%) and kidney weights (−15%) in comparison to the recovery control group was recorded. The Panel considered the changes in organ weights as not toxicologically relevant as they were only observed in one sex (relative weights of the liver, thymus, heart, kidney, spleen), in the absence of a dose–response relationship (relative weights of the liver and the thymus), no consistency between the direction of the changes at the end of the treatment and after the recovery period (the relative weight of ovaries), only recorded at the end of the recovery period (spleen), a low magnitude of the change (the relative liver weight), no statistically significant changes in the absolute weights and no gross pathological and histopathological changes in organs and tissues were observed.

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

The Panel identified a no observed adverse effect level (NOAEL) of 794 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity[39]

The allergenicity assessment considers 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 *A. luchuensis* strain FLOSC 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.[40]

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

Pectate lyases present in plant tissues/or pollen are reported for their role in allergenicity, including the oral allergy syndrome. In Mediterranean areas, *Cupressus sempervirens* (Italian cypress or Mediterranean cypress) is the most common pollinating species. Pectate lyase from the cypress tree was identified as a group 1 major allergen (Charpin et al., 2019). No matches were found of the enzyme under assessment and lyases from pollen. A link to the oral allergy syndrome based on ingestion of allergens cross-reacting with pollen is not likely.

Occupational allergic asthma due to pectinase was documented by Hartmann et al. (1983), Sen et al. (1998), Kuske et al. (2018) and Belleri et al. (2002). However, several studies have shown that adults with occupational asthma to an enzyme can commonly ingest the corresponding respiratory allergens without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Brisman, 2002; Poulsen, 2004; Armentia et al., 2009).

According to the information provided, substances or products that may cause allergies or intolerances (Regulation (EU) No 1169/2011[41]) are used as raw materials ([redacted]). In addition, [redacted] known allergen, is also present in the media fed to

[39] Technical dossier/Additional information, 7 June 2021/Annex 4.

[40] Technical dossier/3.2. Risk Assessment Data/p. 51–52; Technical dossier/Annex L.

[41] 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.

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 considers that potentially allergenic residues of these materials employed as protein sources are not expected to be present in the food enzyme.

The Panel considers that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme cannot be excluded, but the likelihood of such reactions occurring is considered to be low.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in fruit and vegetable processing for juice production[42] at the recommended use levels up to 6.25 mg TOS/kg fruit or vegetable mash.[43]

In fruit and vegetable processing for juice production, the food enzyme is added to a mash of fruits or vegetables (with or without peels)[44], where the pectin lyase cleaves galacturonan-rich cell wall components (e.g. pectin, in particular, highly esterified pectin) to facilitate the release of juice. The enzymatic treatment can lead to higher yields. By using this food enzyme several types of juices can by produced, ready to drink, concentrated and dehydrated juices.[42]

The food enzyme remains in the processed juices. The survival of the activity will depend on the food process conditions.[45]

3.5.2. Dietary exposure estimation

Chronic exposure was calculated by combining the maximum recommended use level provided by the applicant with the individual data from the EFSA Comprehensive European Food Consumption Database. The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP Panel, 2021b). Exposure from individual FoodEx categories was subsequently summed up, averaged over the total survey period 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 one day per subject were excluded and high-level exposure/intake was calculated for only those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011).

Table 2 provides an overview of the derived exposure estimates across all surveys. Detailed average 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 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). The highest dietary exposure at the 95th percentile to the food enzyme–TOS was estimated to be 0.268 mg TOS/kg bw per day in children 3–9 years of age.

[42] Technical dossier/Additional data, June 2021.
[43] Technical dossier/3.2. Risk assessment data/p. 39/Table 3.2.1.4-1.
[44] Technical dossier/3.2. Risk Assessment Data/p. 38/Figure 3.2.1.4.1-1.
[45] Technical dossier/3.2. Risk Assessment Data/p. 38/Figure 3.2.1.4-1; Technical dossier/3.3. Risk Management Data.

Table 2: 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)	0.002–0.040 (11)	0.011–0.151 (15)	0–0.085 (19)	0.001–0.047 (21)	0.002–0.028 (22)	0.001–0.017 (22)
Min–max 95th percentile (number of surveys)	0–0.158 (9)	0.068–0.253 (13)	0.002–0.268 (19)	0.003–0.161 (20)	0.015–0.117 (22)	0.004–0.078 (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 3.

Table 3: Qualitative evaluation of the influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction of impact
Model input data	
Consumption data: different methodologies/representativeness/underreporting/ misreporting/no portion size standard	+/–
Use of data from food consumption surveys of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Possible national differences in categorisation and classification of food	+/–
Model assumptions and factors	
FoodEx categories included in the exposure assessment were assumed to always contain the food enzyme–TOS	+
Exposure to food enzyme–TOS was always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
Use of recipe fractions in disaggregation FoodEx categories	+/–
Use of technical factors in the exposure model	+/–

TOS: total organic solids.
+: 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.

3.6. Margin of exposure

A comparison of the NOAEL (794 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0–0.151 mg TOS/kg bw per day at the mean and from 0 to 0.268 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposure (MOE) of at least 2,963.

4. Conclusions

Based on the data provided, and the derived margin of exposure, the Panel concluded that the food enzyme pectin lyase produced with a genetically modified strain of *A. luchuensis* strain FLOSC does not give rise to safety concerns under the intended conditions of use.

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

5. Documentation as provided to EFSA

Technical dossier 'Application for authorisation of pectin lyase produced from genetically modified *Aspergillus niger* agg. (strain FLOSC) in accordance with Regulation (EC) No 1331/2008'. 19 January 2015. Submitted by Advanced Enzyme Technologies LtD.

Additional information. 7 June 2021. Submitted by Advanced Enzyme Technologies LtD.

Summary report on technical data and dietary exposure related to pectin lyase from a strain of *Aspergillus niger* agg. (strain FLOSC) by Advanced Enzyme Technologies. February 2016. Delivered by Hylobates Consulting and BiCT (Rome, Italy).

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Abbreviations

ALT	alanine aminotransferase
AMFEP	Association of Manufacturers and Formulators of Enzyme Products
AST	aspartate aminotransferase
[redacted]	[redacted]
Bp	base pair
Bw	body weight
CAS	Chemical Abstracts Service
EFSA CEF Panel	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
EFSA CEP Panel	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EFSA GMO Panel	EFSA Panel on Genetically Modified Organisms
[redacted]	[redacted]
DNA	Deoxyribonucleic acid
EC	European Commission
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
FoodEx	the food classification and description system
GGT	gamma glutamyl transferase
GLP	Good Laboratory Practice
GM	genetically modified
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LOD	limit of detection
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MOE	margin of exposure
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
PLU	unit of pectin lyase
RBC	red blood cells
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
TOS	total organic solids
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.2022.7235#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 one 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, 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, 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, Italy, Latvia, Netherlands, Portugal, 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).