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Safety evaluation of the food enzyme pectinesterase from the genetically modified *Aspergillus luchuensis* strain FLZSC

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Abstract

The food enzyme pectinesterase (pectin pectylhydrolase, EC 3.1.1.11) is produced with the genetically modified *Aspergillus luchuensis* strain FLZSC by Advanced Enzyme Technologies Ltd. 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 fruit and vegetable processing for the production of juices and other fruit or vegetable products, as well as in the manufacture of alcoholic beverages from fruits other than grapes. Dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.274 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not raise a safety concern. The systemic toxicity was assessed by a repeated dose 90-day oral toxicity study in rats. The Panel identified a no observed adverse effect level of 833 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 3,040. A search was made for the similarity of the amino acid sequence of the food enzyme to those of known allergens and three matches with respiratory allergens were found. The Panel considered that under the intended conditions of use, the risk of allergic reactions by dietary exposure, particularly in individuals sensitised to olive pollen, although unlikely, cannot be excluded. The Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

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Keywords: food enzyme, pectinesterase, pectin pectylhydrolase, EC 3.1.1.11, pectin methylesterase, *Aspergillus luchuensis*, 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 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 European Union (EU) Community list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7(2) of Regulation (EC) No 1332/2008 on food enzymes.

Five applications have been introduced by the companies "Roquette", "Novozymes A/S", "DSM Food Specialities B.V" and "Advanced Enzyme Technologies Ltd." for the authorisation of the food enzyme Beta-amylase from wheat (*Triticum* spp.), Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-AN), Chymosin from a genetically modified strain of *Kluyveromyces lactis* (strain CIN), Polygalacturonase from a genetically modified strain of *Aspergillus niger* (strain FLYSC) and Pectinesterase from a genetically modified strain of *Aspergillus niger* (strain FLZSC).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008, the Commission has verified that the 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, 11.03.2011, pp. 15–24.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes Beta-amylase from wheat (*Triticum* spp.), Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-AN), Chymosin from a genetically modified strain of *Kluyveromyces lactis* (strain CIN), Polygalacturonase from a genetically modified strain of *Aspergillus niger* (strain FLYSC) and Pectinesterase from a genetically modified strain of *Aspergillus niger* (strain FLZSC) 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 Pectinesterase from a genetically modified *Aspergillus niger* strain FLZSC.

Recent data identified the production microorganism as *Aspergillus luchuensis* (Section 3.1). Therefore, this name will be used in this opinion instead of *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 pectinesterase from a genetically modified *Aspergillus niger* (strain FLZSC).

Additional information was requested from the applicant during the assessment process on 24 March 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) 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	Pectinesterase
Systematic name	Pectin pectylhydrolase
Synonyms	Pectin methylesterase, pectin methoxylase, pectin demethoxylase
IUBMB No	EC 3.1.1.11
CAS No	9025-98-3
EINECS No	232-807-0

Pectinesterases catalyse the hydrolysis of the methylated carboxyl groups of pectin, resulting in the generation of pectic acid and methanol. The enzyme under assessment is intended to be used in fruit and vegetable processing for the production of juices and other fruit or vegetable products, as well as in the manufacture of alcoholic beverages from fruits other than grapes.

3.1. Source of the food enzyme

The pectinesterase is produced with the genetically modified filamentous fungus *Aspergillus luchuensis* (formerly *Aspergillus acidus*) strain FLZSC, which is deposited at the American Type Culture Collection (ATCC, USA), with deposit number [redacted].[4] The production strain was identified as *A. luchuensis* by [redacted].[5]

[4] Technical dossier/Additional data June 2021/Annex 1.
[5] Technical dossier/1st submission/Annex I1.

3.1.1. Characteristics of the parental and recipient microorganisms

The parental strain is *A. luchuensis* [redacted].

3.1.2. Characteristics of introduced sequences

The sequence encoding the pectinesterase is [redacted].

3.1.3. Description of the genetic modification process

The purpose of genetic modification was [redacted][6]

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* FLZSC differs from the recipient strain [redacted][7]

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[8], with food safety procedures based on hazard analysis and critical control points, and in accordance with current good manufacturing practice.[9]

The production strain is grown as a pure culture using a typical industrial medium in a submerged 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. The filtrate containing the enzyme is then further purified and concentrated, including ultrafiltration and diafiltration in which the enzyme protein is retained, while most of the low molecular mass material passes the filtration membrane and is discarded. Finally, after stabilisation with [redacted], the food enzyme is spray-dried prior to analysis.[10] 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.[11]

[6] Technical dossier/1st submission/Annex M.
[7] Technical dossier/Additional data June 2021/Annex A.
[8] 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.
[9] Technical dossier/3.2. Risk Assessment Data/Annex F.
[10] Technical dossier/3.2. Risk Assessment Data/pg. 31-36/Annex G.
[11] Technical dossier/3.2. Risk Assessment Data/Annex G_Appendix 2.

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 pectinesterase is a single polypeptide chain of 331 amino acids.[12] The molecular mass of the mature protein, calculated from the amino acid sequence, is 35.6 kDa. The food enzyme was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. A consistent protein pattern was observed across all batches.[13] The gels showed three major protein bands corresponding to apparent molecular masses between 29 and 43 kDa, corresponding to different forms of the glycosylated pectinesterase, as shown by zymogram, deglycosylation analysis and mass spectrometry.[14] No other enzymatic activities were reported.[15]

The in-house determination of pectinesterase activity is based on hydrolysis of citrus pectin (reaction conditions: pH 3.5, 30°C, 5 min). The enzymatic activity is determined by measuring the released free carboxylic groups that are titrated with sodium hydroxide. Pectinesterase activity is expressed in Pectin Methyl Esterase Activity Units/g (U/g). One U is defined as the amount of the enzyme that will release 1 μmol of acid groups per minute.[16]

The food enzyme has a temperature optimum around 55°C (pH 3.5) and a pH optimum around pH 5.0 (30°C). Thermostability was tested after pre-incubation of the food enzyme for 120 min at different temperatures (pH 3.5). The activity decreased above 60°C, showing no residual activity above 70°C.[17]

3.3.2. Chemical parameters

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).[18] The mean total organic solids (TOS) of the three food enzyme batches is 77.9% and the mean enzyme activity/TOS ratio is 110.1 U/mg TOS.

Table 1: Composition of the food enzyme preparation

Parameters	Unit	Batches		
		1	2	3[(a)]
Pectinesterase activity	U/g[(b)] batch	86,124	89,178	82,142
Protein	%	47.7	49.8	45.2
Ash	%	9.2	9.0	10.0
Water	%	7.3	6.6	8.0
[redacted] **(excipient)**	%	4.8	6.4	5.0
Total organic solids (TOS)[(c)]	%	78.7	78.0	77.0
Pectinesterase activity/mg TOS	U/mg TOS	109.5	114.3	106.6

(a): Batch used for the toxicological studies.
(b): UNIT: U/g (see Section 3.3.1).
(c): TOS calculated as 100% – % water – % ash – % excipient.

3.3.3. Purity

The lead content in the three batches was below 0.25 mg/kg[19] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing

[12] Technical dossier/3.2. Risk Assessment Data/pg. 5.
[13] Technical dossier/Additional data June 2021/p.7–8.
[14] Technical dossier/3.2. Risk Assessment Data/pg. 4/Annex B.
[15] Technical dossier/3.2. Risk Assessment Data/pg. 9.
[16] Technical dossier/3.2. Risk Assessment Data/pg. 9; Annexes: C and A2.
[17] Technical dossier/3.2. Risk Assessment Data/pg. 11–12; Annex C.
[18] Technical dossier/3.2. Risk Assessment Data/pg. 4, 6, 54; Annexes: A3 and J.
[19] Technical dossier/3.2. Risk Assessment Data/pg. 6/Annexes: A1 and D.

(FAO/WHO, 2006). In addition, the levels of arsenic, cadmium and mercury were below the limits of detection (LoD) of the employed methods.[20,21]

The food enzyme preparation 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.[23]

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, fumonisin B1, ochratoxin A, T-2 toxin, HT2-toxin, zearalenone, deoxynivalenol, ergocornine, ergocristine, ergocryptine, ergometrine, ergosine and ergotamine was examined in the three food enzyme batches and all were below the LoD of the applied methods.[24,25] Any 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

The absence of viable cells of the production strain in the food enzyme was demonstrated in three independent batches analysed in triplicate. [redacted] No colonies were produced.[26]

The absence of recombinant DNA in the food enzyme was demonstrated by PCR analysis of three batches in triplicate. No DNA was detected with primers that would amplify [redacted], with an LoD of 1 ng spiked DNA/g food enzyme.[27]

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 3 (Table 1), which was produced according to the procedure used for commercial production and is 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 Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997a) and following good laboratory practice (GLP).[28]

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 in triplicate were carried out using five concentrations of the food enzyme from 61.72 to 5,000 μg/plate (corresponding to 47.52, 142.6, 427.77, 1,283.3, 3,850 μg TOS/plate). No cytotoxicity was observed at any concentration of the test substance. Upon treatment with the food enzyme, there was no significant increase in revertant colony numbers above the control values in any strain with or without S9-mix.

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

[20] Technical dossier/3.2. Risk Assessment Data/Annex D.
[21] LoDs: Pb, As, Cd = 0.25 mg/kg each; Hg = 0.025 mg/kg.
[22] Technical dossier/3.2. Risk Assessment Data/pg. 6, 9; Annexes: A1, A3.
[23] Technical dossier/3.2. Risk Assessment Data/pg. 6, 9; Annexes: A1, A3, E2.
[24] Technical dossier/3.2. Risk Assessment Data/Annexes: A1, E1, I1.
[25] LoDs: aflatoxins (B1, B2, G1, G2, M1) = 1 μg/kg each; fumonisin B1 = 100 μg/kg; ochratoxin A = 1 μg/kg; T-2 toxin = 10 μg/kg; HT2-toxin = 50 μg/kg; zearalenone = 5 μg/kg; deoxynivalenol = 25 μg/kg; ergocornine, ergocristine, ergocryptine, ergometrine, ergosine, ergotamine = 100 μg/kg each.
[26] Technical dossier/1st submission/Annex M/Appendix 1.3.
[27] Technical dossier/Additional data June 2021/Annexes B and C.
[28] Technical dossier/Annex J/p. 1–52.

3.4.1.2. *In vitro* mammalian chromosomal aberration test

The *in vitro* mammalian chromosomal aberration test was carried out in human peripheral blood lymphocytes according to OECD Test Guideline 473 (OECD, 1997b) and following GLP.[29]

A cytotoxicity study was performed at concentrations ranging from 312 to 5,000 μg/mL, and no inhibition of mitotic activity by 50% or more was observed. Based on these results, two separate experiments were carried out in duplicate cultures. In the first experiment, the cells were exposed to the food enzyme at 1,250, 2,500 and 5,000 μg/mL (corresponding to 962.5, 1,925 and 3,850 μg TOS/mL) in the short-term treatment (4 hours followed by 20 hours recovery period) with and without metabolic activation (S9-mix). In the second experiment, the cells were exposed to the food enzyme at 1,250, 2,500 and 5,000 μg/mL (corresponding to 962.5, 1,925 and 3,850 μg TOS/mL) in the short-term treatment with S9-mix and in the continuous treatment (24 hours) in the absence of S9-mix.

No cytotoxicity was observed at any concentration of the test substance. 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.

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.[30] Groups of 10 male and 10 female Sprague–Dawley rats received by gavage the food enzyme at 250, 500 and 1,000 mg/kg bw per day, corresponding to 208, 417 and 833 mg TOS/kg bw per day. Controls received the vehicle (distilled water). Two recovery groups of six male and six female rats each were treated with 0 or 833 mg TOS/kg bw per day for 90 days followed by a 4-week recovery period.

No mortality was observed.

Functional observation battery tests revealed statistically significant differences to controls in the motor activity; the motor activity was increased in mid-dose males for the first interval (+44%), decreased in mid- and high-dose females for the first interval (−40% and −28%) and decreased in mid-dose females for the second interval (−37%). As these changes were not dose dependent, they were considered not to be of toxicological relevance.

The haematological investigation revealed statistically significant increases in haemoglobin concentration (Hb, low- and mid-dose males; +11% and +11%), total red blood cell (RBC) count (mid- and high-dose males; +10% and +12%), haematocrit (HCT, all treated males; +13%, +12% and +12%), mean corpuscular volume (MCV, low-dose males; +6%), platelet count (all treated females; +23%, +17% and +22%) and a statistically significant decrease in total mean corpuscular haemoglobin concentration (MCHC, high-dose males, −4%). At the end of the recovery period, a statistically significant increase in total MCHC (+2.5%) and total white blood cell (WBC) count (high-dose recovery males; +33%), haemoglobin (+16%), total RBC count (+17%), haematocrit (+14%) and MCHC (+1.6%) (high-dose recovery females) were recorded. All the changes in haematology parameters were considered by the Panel as not toxicologically relevant because the differences were small (males: Hb, HCT, MCV) and/or without a dose–response relationship (males: Hb, HCT, MCV, MCH; females: platelets), or were only present at the end of the recovery period (males: WBC; females: Hb, RBC, HCT, MCHC).

The clinical chemistry investigation revealed statistically significant increases in phosphorous (all treated males; +13%, +26% and +15%, and low-dose females; +19%), globulin (high-dose males; +7%), creatinine (low- and mid-dose males; +32% and +36%), aspartate aminotransferase (AST, low- and mid-dose females; +35% and +31%) and chloride (all treated females; +5.4%, +4% and +3.6%). In addition, a statistically significant decrease was observed in albumin (high-dose males; −24%), calcium (low- and high-dose females; −4% and −3%), bilirubin (low-dose females, −21%) and sodium (low-dose females, −1.8%). At the end of the recovery period, a statistically significant increase in alkaline phosphatase (high-dose recovery males; +63%), albumin (high-dose recovery females; +39%) and a statistically significant decrease in total protein (−19%), blood urea nitrogen (−24%), urea (−24%), alanine aminotransferase (−15%), AST (−61%), calcium (−14%), bilirubin (−64%), globulin (−19%) and cholesterol (−24%), all in high-dose recovery males were recorded.

[29] Technical dossier/Annex J/p. 53–113.
[30] Technical dossier/Annex J/p. 114–647.

The Panel considered the changes at the end of the treatment as not toxicologically relevant as they were only observed in one sex (all parameters), there was no dose–response relationship (phosphorous, creatinine, AST, chloride, calcium, sodium and bilirubin) and the changes were small (globulin, AST, chloride, calcium and sodium). The Panel considered the changes at the end of recovery as not toxicologically relevant as they were not seen at the end of the treatment (all parameters except for globulin) and they were only observed in one sex (all parameters).

Statistically significant changes in organ weights were limited to an increase in the relative weight of thymus in mid-dose females (+44%) when compared with that of controls. The Panel considered this change as not toxicologically relevant as there was no dose–response relationship, the change was only observed in one sex and there were no histopathological changes in the thymus.

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

The Panel identified the no observed adverse effect level (NOAEL) of 833 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity

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

The potential allergenicity of the pectinesterase produced with the genetically modified *A. luchuensis* strain FLZSC 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, three matches were found.[31] The matching allergens were Sal k1.0301 and Sal k1.0201, a pectinesterase from *Salsola kali* (Russian thistle) and Ole e 11, a pectinesterase from *Olea europaea* (olive tree), known as respiratory allergens.

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

Pectinesterases present in plant tissues and pollen are reported for their role in allergenicity: the allergen Ole e 11, a pectinesterase from Olive tree (*Olea europaea*) was identified as a source of allergy (Salamanca et al., 2010), as well as Sal k 1, a pectinesterase from Russian thistle (*Salsola kali*) (Barderas et al., 2007). The Panel noted that oral allergy syndrome, i.e. allergic reactions mainly in the mouth, and seldomly leading to anaphylaxis, is associated with sensitisation to olive pollen.

[redacted] (listed in the Regulation (EU) No 1169/2011[32]) are used as raw materials in the medium fed to the microorganisms. In addition, [redacted], a known source of allergen, is also present. However, during the fermentation process, these products will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the fungal biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that no potentially allergenic residues are present in the food enzyme.

The Panel considered that, under the intended conditions of use, the risk of allergic reactions upon dietary exposure to this food enzyme, particularly in individuals sensitised to olive pollen, although unlikely, 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 three food manufacturing processes at the recommended use levels summarised in Table 2.

[31] Technical dossier/3.2. Risk Assessment Data/p. 55-56/Annex L.

[32] 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.

Table 2: Intended uses and recommended use levels of the food enzyme as provided by the applicant[33]

Food manufacturing process[(a)]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[(b)]
Fruit and vegetable processing for juice production	Fruits and vegetables	2.08–**5.20**
Fruit and vegetable processing for products other than juices (firmed fruit and vegetables)	Fruits and vegetables	5.21–**10.42**
Manufacture of alcoholic beverages from fruits other than grapes	Apple or pear	2.08–**5.20**

(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 juice production, the food enzyme is added to the crushed mash, where the pectinesterase de-esterifies pectins, enabling the action of polygalacturonases.[34] The resulting breakage of the gel structure reduces the viscosity, facilitating the release of juice. The food enzyme–TOS remains in the final juices.

For making firmed fruit and vegetables, the food enzyme is added to sliced pieces during the soaking step.[35] The addition of pectinesterase to fruit pieces aids the depolymerisation of the pectin constitutes of cell wall matrix, consequently facilitating the softening of the fruit and vegetables, which are then made into different food products.[36] The food enzyme–TOS remains in the final foods.[37]

In cider manufacturing, the food enzyme is added after the crushing/milling steps to facilitate the release of juice. It is also added to the cloudy juices to reduce cloud formation and viscosity.[38] The food enzyme TOS remains in the final alcoholic beverages.

Based on data provided on thermostability (see Section 3.3.1), it is expected that the food enzyme is inactivated during cider production and in firmed fruit and vegetable products but may remain active in juices depending on the pasteurisation conditions.

3.5.2. Dietary exposure estimation

Chronic exposure to the food enzyme–TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSA CEP Panel, 2021a). The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP Panel, 2021b). Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period (days) and normalised for body weight. This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only 1 day per subject were excluded and high-level exposure/intake was calculated for only those population groups in which the sample size was sufficiently large to allow the 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 0.274 mg TOS/kg bw per day in infants at the 95th percentile.

[33] Technical dossier/3.2. Risk Assessment Data/Section 3.2.1.4; Additional data June 2021.
[34] Technical dossier/3.2. Risk Assessment Data/Figure 3.2.1.4–1.
[35] Technical dossier/3.2. Risk Assessment Data/Figure 3.2.1.4–2.
[36] Additional data June 2021/Answer 11a and 11b.
[37] Additional data June 2021/Answer 11c.
[38] Technical dossier/3.2. Risk Assessment Data/Figure 3.2.1.4–3.

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)	0.008–0.145 (11)	0.028–0.166 (15)	0.016–0.111 (19)	0.005–0.060 (21)	0.003–0.039 (22)	0.001–0.032 (22)
Min–max 95th (number of surveys)	0.033–0.274 (9)	0.111–0.254 (13)	0.058–0.230 (19)	0.022–0.161 (20)	0.020–0.118 (22)	0.006–0.089 (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	+
Only firmed fruit and vegetable products were indicated by the applicant, but the calculation included also other types of products (e.g. puree)	+
Use of recipe fractions in disaggregation FoodEx categories	+/–
Use of technical factors in the exposure model	+/–

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

The conservative approach applied to the exposure estimate to food enzyme–TOS, in particular assumptions made on the occurrence and use levels of this specific food enzyme, is likely to have led to an overestimation of the exposure.

3.6. Margin of exposure

A comparison of the NOAEL (833 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0.001–0.166 mg TOS/kg bw per day at the mean and from 0.006–0.274 mg TOS/kg bw per day at the 95th percentile resulted in a margin of exposure (MoE) of at least 3,040.

4. Conclusions

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

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

5. Documentation as provided to EFSA

Application for authorisation of Pectinesterase produced from genetically modified *Aspergillus niger* agg. (strain FLZSC) in accordance with Regulation (EC) No 1331/2008. December 2014. Submitted by Advanced Enzyme Technologies Ltd.

Additional information. June 2021. Submitted by Advanced Enzyme Technologies Ltd.

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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 Organisation of the United Nations
GLP	good laboratory practice
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LoD	limit of detection
MoE	margin of exposure
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
SDS-PAGE	sodium dodecyl sulfate-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.2022.7674#support-information-section).

The file contains two sheets, corresponding to two tables.

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

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

Appendix B – Population groups considered for the exposure assessment

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