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Safety evaluation of the food enzyme containing endo-polygalacturonase, pectinesterase, pectin lyase and non-reducing end α-L-arabinofuranosidase activities from the *Aspergillus niger* strain PEC

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Abstract

The food enzyme has four declared activities (endo-polygalacturonase ((1→4)-α-D-galacturonan glycanohydrolase (endo-cleaving); 3.2.1.15), pectinesterase (pectin pectylhydrolase; 3.1.1.11), pectin lyase ((1→4)-6-*O*-methyl-α-D-galacturonan lyase; 4.2.2.10) and non-reducing end α-L-arabinofuranosidase (α-L-arabinofuranoside non-reducing end α-L-arabinofuranosidase; 3.2.1.55) and is produced with the non-genetically modified *Aspergillus niger* strain PEC by DSM Food Specialties B.V. The food enzyme is free from viable cells of the production organism. The food enzyme is intended to be used in the manufacture of alcoholic beverages from fruits other than grapes, fruit and vegetable processing for juice production, and wine and wine vinegar production. Dietary exposure was estimated to be up to 0.25 mg TOS/kg bodyweight (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 204 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 800. A search for similarity of the amino acid sequence of the food enzyme to known allergens was made and several matches were 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, particularly for individuals sensitised to several pollen allergens or papaya allergens. Based on the data provided, the Panel concluded that this food enzyme did not give rise to safety concerns, under the intended conditions of use.

Keywords: endo-polygalacturonase, 3.2.1.15, pectinesterase, 3.1.1.11, pectin lyase, 4.2.2.10, non-reducing end α-L-arabinofuranosidase, 3.2.1.55, *Aspergillus niger*, non-genetically modified microorganism

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1. Introduction

Article 3 of the Regulation (EC) No 1332/2008[1] provides definition for 'food enzyme' and 'food enzyme preparation'.

'Food enzyme' means a product obtained from plants, animals or micro-organisms or products thereof including a product obtained by a fermentation process using micro-organisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.

'Food enzyme preparation' means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

Before January 2009, food enzymes other than those used as food additives were not regulated or were regulated as processing aids under the legislation of the Member States. On 20 January 2009, Regulation (EC) No 1332/2008 on food enzymes came into force. This Regulation applies to enzymes that are added to food to perform a technological function in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food, including enzymes used as processing aids. Regulation (EC) No 1331/2008[2] established the European Union (EU) procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

- it does not pose a safety concern to the health of the consumer at the level of use proposed;
- there is a reasonable technological need;
- its use does not mislead the consumer.

All food enzymes currently on the European Union market and intended to remain on that market, as well as all new food enzymes, shall be subjected to a safety evaluation by the European Food Safety Authority (EFSA) and approval via an EU Community list.

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

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

1.1.1. Background as provided by the European Commission

Only food enzymes included in the 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 Association of Manufacturers and Formulators of Enzyme Products (AMFEP), and by the companies "DSM Food Specialties B.V" and "Novozymes A/S" for the authorisation of the food enzymes Pectinase, Poly-galacturonase, Pectin esterase, Pectin lyase and Arabanase from *Aspergillus niger*, Phospholipase A_2 from a genetically modified strain of *Aspergillus niger* (strain PLA), Pectinesterase from a genetically modified strain of *Aspergillus niger* (strain PME), Endo-1,4-β-xylanase from a genetically modified strain of *Aspergillus niger* (strain XEA) and Maltogenic amylase produced by a genetically modified strain of *Bacillus subtilis* (strain NZYM-SO), respectively.

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 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, p. 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 Pectinase, Poly-galacturonase, Pectin esterase, Pectin lyase and Arabanase from *Aspergillus niger*, Phospholipase A_2 from a genetically modified strain of *Aspergillus niger* (strain PLA), Pectinesterase from a genetically modified strain of Aspergillus niger (strain PME), Endo-1,4-β-xylanase from a genetically modified strain of *Aspergillus niger* (strain XEA) and Maltogenic amylase produced by a genetically modified strain of *Bacillus subtilis* (strain NZYM-SO) 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 the food enzyme pectinase, poly-galacturonase, pectin esterase, pectin lyase and arabanase from *A. niger* submitted by AMFEP.

The application was submitted initially as a joint dossier[4] and identified as the EFSA-Q-2015-00038, EFSA-Q-2015-00039, EFSA-Q-2015-00040, EFSA-Q-2015-00041 and EFSA-Q-2015-00042. During the risk assessment phase, it was found that the technical dossier was too generic to be evaluated. A solution was found on 16 March 2020 via an ad hoc meeting between EFSA, the European Commission and representatives from the Association of Manufacturers and Formulators of Enzyme Products (AMFEP).[5] It was agreed that joint dossiers will be split into individual data packages.

The current opinion addresses one data package originating from the joint dossier EFSA-Q-2015-00038, EFSA-Q-2015-00039, EFSA-Q-2015-00040, EFSA-Q-2015-00041 and EFSA-Q-2015-00042. This data package, identified as EFSA-Q-2021-00587, concerns the food enzyme pectinase, poly-galacturonase, pectin esterase, pectin lyase and arabanase that is produced with a strain of *A. niger* and submitted by DSM Food Specialities B.V.

2. Data and methodologies

2.1. Data

The applicant has submitted a data package in support of the application for authorisation of the food enzyme pectinase from a *A. niger* strain PEC. The dossier was updated on 15 October 2021.

Additional information was requested from the applicant during the assessment process on 13 December 2021 and received on 14 March 2022 (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 existing guidance documents of EFSA Scientific Committee.

The current '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) has been followed for the evaluation of the application, with the exception of the exposure assessment, which was carried out in accordance to the methodology described in the CEF Panel 'Statement on the exposure assessment of food enzymes' (EFSA CEF Panel, 2016).

3. Assessment

The food enzyme is a pectinolytic complex containing four declared activities which the applicant collectively describes as 'pectinase':

IUBMB nomenclature	Endo-polygalacturonase
Systematic name	(1→4)-α-D-galacturonan glycanohydrolase (endo-cleaving)
Synonyms	Pectinase, pectin depolymerase, pectolase, pectin hydrolase

[4] Commission Implementing Regulation (EU) No 562/2012 of 27 June 2012 amending Commission Regulation (EU) No 234/2011 with regard to specific data required for risk assessment of food enzymes Text with EEA relevance OJ L 168, 28.6.2012, p. 21–23.

[5] The full detail is available at the https://www.efsa.europa.eu/en/events/event/ad-hoc-meeting-industry-association-amfep-joint-dossiers-food-enzymes

IUBMB nomenclature	Endo-polygalacturonase
IUBMB No	3.2.1.15
CAS No	9032-75-1
EINECS No	232-885-6

Endo-polygalacturonases catalyse the random hydrolysis of (1→4)-α-D-galactosiduronic linkages of pectin and other polygalacturonans, resulting in the generation of partially hydrolysed galacturonan.

IUBMB nomenclature	Pectinesterase
Systematic name	Pectin pectylhydrolase
Synonyms	Pectin demethoxylase; pectin methoxylase; pectin methyl esterase
IUBMB No	3.1.1.11
CAS No	9025-98-3
EINECS No	232-807-0

Pectinesterases catalyse the hydrolysis of methylated carboxyl groups of pectin, resulting in the generation of pectic acid and methanol.

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

Pectin lyases catalyse the β-eliminative cleavage of 1,4-α-D-galactosiduronic linkages of pectin, resulting in the generation of oligosaccharides with 4-deoxy-6-*O*-methyl-α-D-galact-4-enuronosyl groups at their non-reducing ends.

IUBMB nomenclature	Non-reducing end α-L-arabinofuranosidase
Systematic name	α-L-arabinofuranoside non-reducing end α-L-arabinofuranosidase
Synonyms	α-Arabinosidase; α-arabinofuranosidase; α-L-arabinanase
IUBMB No	3.2.1.55
CAS No	9067-74-7
EINECS No	232-957-7

Non-reducing end α-L-arabinofuranosidases catalyse the hydrolysis of terminal non-reducing α-L-arabinofuranoside residues in α-L-arabinosides, releasing α-L-arabinofuranose.

The food enzyme is intended to be used in the manufacture of alcoholic beverages from fruits other than grapes, fruit and vegetables processing for juice production, and wine and wine vinegar production.

3.1. Source of the food enzyme

The food enzyme is produced with the filamentous fungus *A. niger* strain PEC, which is deposited at the Westerdijk Fungal Biodiversity Institute (the Netherlands), with deposit number [redacted][6] The production strain was identified as *A. niger* [redacted][7] *A. niger* PEC is not genetically modified.

[6] Technical Dossier/1st submission/Annex I-7.
[7] Technical Dossier/1st submission/Annex I-5.

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, fed-batch fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in which enzyme protein is retained, while most of the low molecular mass material passes the filtration membrane and is discarded.[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]

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 protein has three subunits of 362, 384 and 378 amino acids, with molecular masses of 37.7, 40.6 and 39.6 kDa, respectively. The pectinesterase protein has three subunits of 397, 327 and 331 amino acids, with molecular masses of 43.1, 34.6 and 35.6 kDa, respectively. The pectin lyase protein is a single polypeptide chain of 379 amino acids with molecular mass of 39.8 kDa. The non-reducing end α-L-arabinofuranosidase protein has two subunits of 499 and 332 amino acids, with molecular masses of 52.5 and 35.8 kDa, respectively. All molecular masses were calculated from the amino acid sequence.[12]

The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). A consistent protein pattern was observed across all batches. The gels showed several bands migrating between the marker proteins of 36.5 and 55.4 kDa in all batches, consistent with the expected masses of the enzyme subunits. The protein profile also included bands of lesser staining intensity.[12] No other enzyme activities have been reported.[13]

The in-house determination of total 'pectinase activity' is based on degradation of methylated pectin, which is measured viscosimetrically (reaction conditions pH 3.80, 50°C). A calibration curve is constructed with a series of internal enzyme standards and used to quantify the enzyme activity. Enzyme activity is expressed in pectinase Units/g (AVJP/g). One AVJP is defined as the amount of enzyme per 1 mL enzyme solution that causes a change in viscosity of the substrate with a speed giving slope of 0.00027 per minute under the conditions of the test.[14]

The in-house determination of endo-polygalacturonase activity is based on hydrolysis of polygalacturonic acid, which is measured viscosimetrically (reaction conditions pH 4.5, 45°C). A calibration curve is constructed with a series of internal enzyme standards and used to quantify the enzyme activity. Enzyme activity is expressed in endo-Polygalacturonase Units/g (PGU/g). One PGU is defined as the amount of enzyme per 1 mL enzyme solution that creates a line with a slope of 0.068 when the ratio of the viscosity of a blank versus that of the test solution is plotted against the incubation time under the assay conditions.[15]

The in-house determination of pectin lyase activity is based on degradation of the substrate pectin (reaction conditions pH 5.1, 30°C). The enzymatic activity is determined by measuring the formation of unsaturated bonds in the released oligosaccharides spectrophotometrically at 235 nm. Enzyme activity is expressed in Pectin Lyase II Units/g (PEL Units/g). One PEL Unit is defined as the amount of enzyme

[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/1st submission/p. 48.
[10] Technical Dossier/1st submission/pp. 48–54 and Annex I-10.
[11] Technical Dossier/1st submission/p. 51 and Annex I-11.
[12] Technical Dossier/1st submission/pp. 32–34.
[13] Technical Dossier/1st submission/pp. 32–33.
[14] Technical Dossier/1st submission/Annex I-2/pp. 2–13.
[15] Technical Dossier/1st submission/Annex I-2/pp. 14–26.

that releases one μmol of galacturonic acid with an unsaturated bond at its non-reducing end per minute under the assay conditions.[16]

The in-house determination of pectinesterase activity is based on hydrolysis of the ester bonds in methylated pectin (reaction conditions pH 4.5, 30°C). The enzymatic activity is determined by titration of the carboxylic acid groups formed during the reaction. Enzyme activity is expressed in Pectinesterase Units/g (PEU/g). One PEU is defined as the amount of enzyme that hydrolyses 1 μmol of carboxy methylester per minute under the assay conditions.[17]

The in-house determination of non-reducing end α-L-arabinofuranosidase activity is based on hydrolysis of *p*-nitrophenyl-α-L-arabinofuranoside (reaction conditions pH 4.4). The enzymatic activity is determined by measuring the release of *p*-nitrophenol spectrophotometrically at 405 nm. Enzyme activity is expressed in Arabinofuranosidase Units/g (ARF Units/g). One ARF Unit is defined as the amount of enzyme that releases 1 nmol of *p*-nitrophenol per second under the assay conditions.[18]

The 'pectinase', pectin lyase and endo-polygalacturonase activities have a temperature optimum around 50°C (pH 3.8) and a pH optimum around pH 4.5 (50°C). Thermostability was tested after a pre-incubation of the food enzyme for 15 min at different temperatures (pH 3.8). No activity was detected at 75°C and above.[19]

The pectinesterase activity has a temperature optimum from 40 to 45°C (pH 4.5) and a pH optimum around pH 4.5 (30°C). Thermostability was tested after a pre-incubation of the food enzyme for 15 min at different temperatures (pH 4.5). No activity was detected at 66°C and above.[20]

The non-reducing end α-L-arabinofuranosidase activity has a temperature optimum at 65°C (pH 4.4) and a pH optimum around pH 4.5 (37°C). Thermostability was tested after a pre-incubation of the food enzyme for 15 min at different temperatures (pH 4.4). No residual activity was detected at 70°C and above.[21]

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). The mean total organic solids (TOS) of the three food enzyme batches for commercialisation is 20.6%. The mean pectinase activity/TOS ratio is 4,554, the mean endo-polygalacturonase activity/TOS ratio is 16.1 PGU/mg TOS, the mean pectin lyase activity/TOS ratio is 1.9 PEL Unit/mg TOS, the mean pectinesterase activity/TOS ratio is 26.9 PEU/mg TOS, and the mean non-reducing end α-L-arabinofuranosidase activity/TOS ratio is 12.6 ARF Unit/mg TOS.[22]

Table 1: Composition of the food enzyme[(h)]

Parameters	Unit	Batches			
		1	2	3	4[(a)]
Pectinase activity	AVJP Unit/g[(b)]	743,000	1,285,000	801,500	901,000
Endo-polygalacturonase activity	PGU/g batch[(c)]	2,745	4,350	2,855	2,560
Pectin lyase activity	PEL Unit/g batch[(d)]	290	574	356	–
Pectinesterase activity	PEU/g[(e)]	4,465	7,370	4,800	3,220
Non-reducing end α-L-arabinofuranosidase activity	ARF Unit/g[(f)]	1,990	3,110	2,600	2,990
Protein	%	9.5	18.8	11.5	14
Ash	%	1.0	1.3	1.0	2.4
Water	%	82.9	71.3	80.6	77.2
Total organic solids (TOS)[(g)]	%	16.1	27.4	18.4	20.4

[16] Technical Dossier/1st submission/Annex I-2/pp. 27–33.
[17] Technical Dossier/1st submission/Annex I-2/p. 34.
[18] Technical Dossier/1st submission/Annex I-2/pp. 35–48.
[19] Technical Dossier/1st submission/pp. 37–38.
[20] Technical Dossier/1st submission/pp. 39–40.
[21] Technical Dossier/1st submission/pp. 40–42.
[22] Technical Dossier/1st submission/p. 31 and Annex I-3.

Parameters	Unit	Batches			
		1	2	3	4(a)
Pectinase activity/mg TOS	AVJP Unit/mg TOS	4,615	4,690	4,356	4,417
Endo-polygalacturonase Activity/mg TOS	PGU/mg TOS	17.0	15.9	15.5	12.5
Pectin lyase activity/mg TOS	PEL Unit/mg TOS	1.8	2.1	1.9	–
Pectinesterase activity/mg TOS	PEU/mg TOS	27.7	26.9	26.1	15.8
Non-reducing end α-L-arabinofuranosidase activity/mg TOS	ARF Unit/mg TOS	12.4	11.4	14.1	14.7

(a): Batch used for the toxicological studies.
(b): AVJP: Pectinase Unit (see Section 3.3.1).
(c): PGU: Polygalacturonase Unit (see Section 3.3.1).
(d): PEL Unit: Pectin lyase Unit (see Section 3.3.1).
(e): PEU: Pectinesterase Unit (see Section 3.3.1).
(f): ARF Unit: Arabinofuranosidase Unit (see Section 3.3.1).
(g): TOS calculated as 100% - % water - % ash.
(h): Technical Dossier/1st submission/pp. 31, 71 and Annexes I-3, I-17/p. 35.

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[23] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[24,25]

The food enzyme complies with the microbiological criteria (for total coliforms, *Escherichia coli* and *Salmonella*)[23] as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[24] No antimicrobial activity was detected in any of the tested batches (FAO/WHO, 2006).[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 fumonisins and ochratoxin A was examined in the three commercial food enzyme batches and was below the limit of detection (LoD) of the applied methods.[26,27] The presence of aflatoxin B1, T2 toxin, ochratoxin A and zearalenone was examined in the batch for toxicological studies and all were below the LoD of the applied methods.[28,29] 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 of the production strain

The absence of viable cells of the production strain in the food enzyme was demonstrated [redacted] No colonies were produced. In a second analysis, [redacted] No colonies were produced. [redacted]

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

[23] Technical Dossier/1st submission/p. 71 and Annexes I-3, I-17/p. 35.
[24] Technical Dossier/1st submission/p. 13.
[25] LOD: Pb = 5 mg/kg.
[26] LoDs: Ochratoxin A = 1 μg/kg, Fumonisins = 10 μg/kg.
[27] Technical Dossier/1st submission/Annexes I-3 and I-4/p. 79.
[28] LoDs: Aflatoxin B1 = 5 μg/kg, Ochratoxin A = 20 μg/kg, T2 toxin, Zearalenone = 100 μg/kg each.
[29] Technical Dossier/1st submission/Annexes I-16/p. 33.

been provided. The batch 4 (Table 1) used in these studies has similar protein pattern as the batches used for commercialization and lower chemical purity, and, thus, 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, 1997a,b) and following Good Laboratory Practice (GLP).[30] Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *E. coli* WP2uvrA(pKM101) were used in the presence or absence of metabolic activation (S9-mix), applying the treat and plate method. Two separate experiments were carried out using five different concentrations of the food enzyme (100–10,000 μg dry matter/plate, corresponding to 90, 268, 895, 2,684 and 8,947 μg TOS/plate). No cytotoxicity was observed at any concentration level of the test substance. Upon treatment with the food enzyme, there was no biologically relevant increase in revertant colony numbers above the control values in any strain with or without S9-mix.

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

3.4.1.2. *In vitro* mammalian 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.[31]

Two separate experiments were performed in duplicate cultures. In the first experiments, concentrations of the food enzyme ranging from 39.6 to 5,000 μg dry matter/mL (corresponding to 35.5 to 4,473 μg TOS/mL) were tested in a short-term treatment (3 h followed by 19 h recovery period) with and without metabolic activation (S9-mix). In the second experiment, concentrations of the food enzyme ranging from 312.5 to 5,000 μg dry matter/mL (corresponding to 280 to 4,473 μg TOS/mL) were tested in the short-term treatment with S9-mix and in a continuous treatment (19 h) in the absence of S9-mix. In both tests, lymphocytes were scored for chromosomal aberrations at concentrations of 1,250, 2,500 and 5,000 μg dry matter/mL (corresponding to 1,118, 2,237 and 4,473 μg TOS/mL). No cytotoxicity above 30% was seen at any concentration tested with and without S9-mix. The frequency of structural and numerical chromosomal aberrations in treated cultures was not statistically different to the values detected in negative controls and within the range of the laboratory historical control data.

The Panel concluded that the 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 toxicological principles of the US Food and Drug Administration (US FDA, 1982) and following GLP.[32] The study is in accordance with OECD Test Guideline 408 (OECD, 1998) with the following deviations: Detailed clinical observations and functional observations were not performed, blood urea nitrogen and creatinine were not determined, adrenals were not weighed and only one level of the spinal cord was examined in the microscopy. The Panel considered that these deviations are minor and do not impact on the evaluation of the study.

Groups of 20 male and 20 female CD rats received by gavage the food enzyme in doses of 100, 300 or 1,000 mg/kg bw per day corresponding to 20.4, 61.2 or 204 mg TOS/kg bw per day. Controls received the vehicle.

No mortality was observed.

The haematological investigation revealed a statistically significant decrease in the haemoglobin concentration (Hb, −3.7%, −3.7%, −3.7%), in the mean corpuscular haemoglobin (MCH, −2.7%, −4.3%, −3.2%) and in the mean corpuscular volume (MCV, −2.2%, −3.7%, −3.3%) in treated males, a decrease in the haematocrit (Ht) in high-dose males (−2%) and in high-dose females (−4.7%), a decrease in the red blood cell count (RBC, −4.7%) in high-dose females, a decrease in the white blood cell count (WBC, −24%) and in the lymphocyte count (LC, −26%) in low-dose females,

[30] Technical dossier/1st submission/p. 67 and Annex I-16.
[31] Technical dossier/1st submission/p. 68 and Annex I-17.
[32] Technical dossier/1st submission/pp. 51–52 and Annex I-18.

and an increase in the prothrombin time (PT, +9.3%) in mid-dose females. The Panel considered the differences as not toxicologically relevant because the changes were small (Hb, MCH, MCV, Ht, RBC and PT), there was no dose–response relationship (Hb, MCH, MCV, WBC, LC and PT) and the changes were only observed in one sex (Hb, MCH, MCV, RBC, WBC, LC and PT).

The clinical chemistry investigation revealed a statistically significant increase in sodium (+0.7%, +1.4%) in low- and mid-dose males, a decrease in calcium (−6.0%, −3,4%) in mid- and high-dose males, a decrease in γ-glutamyl transpeptidase activity (γ-GGT, −100%) in mid-dose females, a decrease in total protein (TP, −5.6%) in high-dose females, a decrease in potassium (−10%, −7.8%) in low- and mid-dose females, a decrease in calcium (−3.1%) in mid-dose females and an increase in inorganic phosphorus (Phos, +20%) in high-dose females. The Panel considered the differences as not toxicologically relevant because the changes were small (sodium, potassium, calcium and TP), there was no dose–response relationship (sodium, potassium, calcium and γ-GGT) and the changes were only observed in one sex (sodium, potassium, γ-GGT, TP and Phos).

Statistically significant changes in organ weights included a decrease in the relative spleen weight (−9.7%) in low-dose females. The Panel considered this change as not toxicologically relevant because the change was small, there was no dose–response relationship, the change was only observed in one sex and there were no gross or histopathological changes in the spleen.

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

The Panel identified a no observed adverse effect level (NOAEL) of 204 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 endo-polygalacturonase, pectinesterase, pectin lyase and non-reducing end α-L-arabinofuranosidase produced with the non-genetically modified *A. niger* strain PEC 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 of different species: maize (*Zea mays*), bahia grass (*Paspalum notatum*), Japanese cedar (*Cryptomeria japonica*), Mountain cedar (*Juniperus ashei*), common timothy-grass (*Phleum pratens)*, trumpet lily (*Lilium longiflorum*), London plane tree (*Platanus acerifolia*), Japanese cypress (*Chamaecyparis obtusa*), Johnson grass (*Sorghum halepense*), oriental plane (*Platanus orientalis*), Russian thistle (*Salsola kali*) and olive tree (*Olea europaea*), all known as respiratory allergens. In addition, a match with a known oral allergen, namely, ripening-induced endo-polygalacturonase 2 from papaya (*Carica papaya*) was also found.[33]

No information is available on oral and respiratory sensitisation or elicitation reactions of these endo-polygalacturonase, pectinesterase, pectin lyase and non-reducing end α-L-arabinofuranosidase.

The Panel notes that there is a match with an oral allergen from papaya. In addition, the Panel notes that there are matches with several plant pollen allergens that are associated with the oral allergy syndrome, i.e. allergic reactions mainly in the mouth but seldomly leading to anaphylaxis.

According to the information provided, [redacted] a known source of allergens, is present in the media fed to the microorganisms. However, during the fermentation process, this product will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the fungal biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that potentially allergenic residues of these materials employed as protein sources are not expected to be present in the food enzyme.

The Panel considered 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 in particular due to the potential presence of homology sequence with several pollen allergens. In addition, the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme cannot be excluded, particularly in individuals sensitised to papaya proteins, but this risk will not exceed that of papaya consumption.

[33] Technical dossier/1st submission/pp. 72–76 and Annexes I-5, I-20.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

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

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

Food manufacturing process[(a)]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[(b)]
Manufacture of alcoholic beverages from fruits other than grapes	Fruit	0.9–**5.9**
Fruit and vegetable processing for juices production	Apple	0.9–**5.9**
Wine and wine vinegar production	Grapes	0.7–**5.5**

TOS: total organic solids.
(a): The description has been harmonised according to the 'EC working document describing the food processes in which food enzymes are intended to be used' – not yet published at the time of adoption of this opinion.
(b): The numbers in bold were used for calculation.
(c): Technical dossier/1st submission/p. 24.

In the manufacturing of fruit-derived alcoholic beverages, the food enzyme is added to fruit such as apple and pear during maceration. It is also added to the fruit must during clarification and before fermentation.[34] All four enzymatic activities contribute to the degradation of pectin. In turn, this can increase the yield of the fruit must, allow efficient extraction of colour or flavouring compounds. The food enzyme TOS remains in the relevant alcoholic beverages.

In fruit and vegetable processing for juices production, the food enzyme is added to fruit or vegetables during crushing and to the pressed juices during clarification step.[35] All four enzymatic activities contribute to the degradation of pectin. The food enzyme TOS remains in the relevant juices or juice concentrates.

In wine and wine vinegar production, the food enzyme is added to grapes during crushing and maceration.[36] All four enzymatic activities contribute to the degradation of pectin in the cell wall. The food enzyme TOS remains in the wine, as well as in wine vinegars.

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

3.5.2. Dietary exposure estimation

Chronic exposure was calculated using the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEP Panel, 2021a). The calculation combines the maximum recommended use level (see Table 2) with the relevant FoodEx categories (EFSA CEP Panel, 2021b), based on individual consumption data. 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 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 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 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 0.253 mg TOS/kg bw per day in children at the 95th percentile.

[34] Technical dossier/1st submission/pp. 85–87.
[35] Technical dossier/1st submission /pp. 87–88.
[36] Technical dossier/1st submission /pp. 89–90.

Table 3: Summary of estimated dietary exposure to food enzyme–TOS in six population groups

Population group	Estimated exposure (mg TOS/kg body weight per day)					
	Infants	**Toddlers**	**Children**	**Adolescents**	**Adults**	**The elderly**
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0.002–0.038 (11)	0.011–0.143 (15)	0.001–0.081 (19)	0.001–0.045 (21)	0.004–0.032 (22)	0.004–0.025 (22)
Min–max 95th percentile (number of surveys)	0.004–0.149 (9)	0.065–0.239 (13)	0.003–0.253 (19)	0.004–0.152 (20)	0.021–0.116 (22)	0.019–0.082 (21)

TOS: total organic solids.

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	
FoodEx categories included in the exposure assessment were assumed to always contain the food enzyme–TOS	+
Juices from different types of fruit and vegetables are included in the calculation, despite that only apple juices were indicated in the technical dossier	+
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 (204 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates of 0.001–0.143 mg TOS/kg bw per day at the mean and from 0.003–0.253 mg TOS/kg bw per day at the 95th percentile, resulted in a margin of exposure (MoE) of at least 806.

4. Conclusions

Based on the data provided and the derived margin of exposure, the Panel concluded that the food enzyme containing endo-polygalacturonase, pectinesterase, pectin lyase and non-reducing end

α-L-arabinofuranosidase activities produced with the non-genetically modified *A. niger* strain PEC did not give rise to safety concerns under the intended conditions of use.

5. Documentation as provided to EFSA

Pectinase, poly-galacturonase, pectin esterase, pectin lyase and α-L-arabinofuranosidase from *Aspergillus niger*. October 2021. Submitted by DSM Food Specialties B.V. The dossier was updated on 15 October 2021.

Additional information. March 2022. Submitted by DSM Food Specialties B.V.

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Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
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
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LoD	limit of detection
MoE	margin of exposure
OECD	Organisation for Economic Cooperation and Development
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	total organic solids
WGS	whole genome sequence
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.2022.7376#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).