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# Safety evaluation of the food enzyme pectinesterase from the genetically modified *Aspergillus niger* strain PME

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## Abstract

The food enzyme pectinesterase (pectin pectylhydrolase; EC 3.1.1.11) is produced with the genetically modified *Aspergillus niger* strain PME by DSM Food Specialties B.V. The genetic modifications do not give rise to safety concerns. The food enzyme is free from viable cells of the production organism and its recombinant DNA. It is intended to be used in fruit and vegetable processing, for juice production and fruit and vegetable processing for products other than juices. Dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.095 mg TOS/kg body weight (bw) per day in European populations. The toxicity studies were carried out with a xylanase obtained from *A. niger* strain XEA. The Panel considered this food enzyme as a suitable substitute for the pectinesterase to be used in the toxicological studies, because both production strains are derived from the same recipient strain, the location of the inserts is comparable, no partial inserts were present and the production methods are essentially the same. 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 (NOAEL) of 1,852 mg TOS/kg bw per day, the highest dose tested, resulting in a margin of exposure of at least 19,495. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and two matches with pollen allergens were found. The Panel considered that, under the intended conditions of use, the risk of allergic reactions upon dietary exposure to this food enzyme, particularly in individuals sensitised to pollen allergens, cannot be excluded. The Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.



**Keywords:** food enzyme, pectinesterase, pectin pectylhydrolase, EC 3.1.1.11, pectin methylesterase, *Aspergillus niger*, genetically modified microorganism

**Requestor:** European Commission

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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[1] 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 A2 from a genetically modified strain of *Aspergillus niger* (strain PLA), Pectinesterase from a genetically modified strain of *Aspergillus niger* (strain PME), Endo-1,4-B-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 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, 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 A2 from a genetically modified strain of *Aspergillus niger* (strain PLA), Pectinesterase from a genetically modified strain of *Aspergillus niger* (strain PME), Endo-1,4-13-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 food enzyme pectin esterase from a genetically modified strain of *Aspergillus niger* (strain PME).

# 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 esterase from a genetically modified *Aspergillus niger* (strain PME). The dossier was updated on January 2015.

Additional information was requested from the applicant during the assessment process on 15 September 2021, and was consequently provided (see 'Documentation provided to EFSA').

Spontaneous additional information was received from the applicant on May 2020 and May 2023.

## 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 demethoxylase |
| IUBMB No | EC 3.1.1.11 |
| CAS No | 9025-98-3 |
| EINECS No | 232–807-0 |

Pectinesterases catalyse the de-esterification of pectin, resulting in the generation of pectic acid and methanol. The food enzyme under assessment is intended to be used in fruit and vegetable processing for juice production and fruit and vegetable processing for products other than juices.

## 3.1. Source of the food enzyme

The pectinesterase is produced with the genetically modified filamentous fungus *Aspergillus niger* strain PME ([redacted]), which is deposited at the Westerdijk Fungal Biodiversity Institute (the Netherlands), with the deposit number [redacted].[4] The production strain was identified as *A. niger*

[4] Technical dossier/Spontaneous data submission May 2020/Safe Deposit certificate.

by [redacted].[5]

### 3.1.1. Characteristics of the parental and recipient microorganisms[6]

The strain *A. niger* [redacted], considered as the parental strain, was developed [redacted] (Van Dijck et al., 2003). [redacted] (Pel et al., 2007; Andersen et al., 2011). [redacted].

The recipient strain *A. niger* [redacted] was developed [redacted]

### 3.1.2. Characteristics of introduced sequences

The sequence encoding the pectinesterase [redacted] derives from *A. niger*. [redacted][7] [redacted][8] [redacted]

### 3.1.3. Description of the genetic modification process

[redacted][9] [redacted].[10]

### 3.1.4. Safety aspects of the genetic modification

The technical dossier contains all necessary information on the recipient microorganism, the donor organism and the genetic modification process.

The production strain *A. niger* PME differs from the recipient strain only in its capacity to overproduce pectinesterase. [redacted].[5]

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

[5] Technical dossier/Additional data June 2022/Annex 1.
[6] Technical dossier/Annex II-3.
[7] Technical dossier/Annex II-6 and II-8.
[8] Technical dossier/Annex II-7 and II-9.
[9] Technical dossier/Annex II-11.
[10] Technical dossier/Annex II-12.

## 3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[11], with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current good manufacturing practice.[12]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, fed-batch fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration. 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.[13] The applicant provided information on the identity of the substances used to control the fermentation and in the subsequent downstream processing of the food enzyme.[14]

The Panel considered that sufficient information has been provided on the manufacturing process and the quality assurance system implemented by the applicant to exclude issues of concern.

## 3.3. Characteristics of the food enzyme

### 3.3.1. Properties of the food enzyme

The pectinesterase is a single polypeptide chain of 331 amino acids.[15] The molecular mass of the mature protein, calculated from the amino acid sequence, is 35.6 kDa.[16] The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.[17] A consistent protein pattern was observed across all batches, showing two major protein bands, the first corresponding to an apparent mass of about 36 kDa, consistent with the expected mass of the enzyme. The second band, corresponding to an apparent mass of 40 kDa, was ascribed to a glycosylated form of the pectinesterase. The protein profile also included bands of lesser staining intensity. No other enzyme activities were reported.

The in-house determination of pectinesterase activity is based on the demethylation of pectin (reaction conditions: pH 4.5, 30°C). The enzymatic activity is determined by measuring the released free carboxylic groups that are titrated with sodium hydroxide. The enzyme activity is expressed in Pectin Esterase Units (PEU)/g. One PEU is defined as the amount of enzyme that hydrolyses 1 μmol of carboxymethylester per minute under the assay conditions.[18]

The food enzyme has a temperature optimum around 45°C (pH 4.5) and a pH optimum around pH 4.5 (30°C).[19] Thermostability was tested after a pre-incubation of the food enzyme for different times and temperatures (pH 4.5). Pectinesterase activity decreased above 55°C, showing no residual activity after 2 min of pre-incubation at 70°C.[20]

### 3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (Table 1).[21] The mean total organic solids (TOS) was 25.9% and the mean enzyme activity/TOS ratio was 551 PEU/mg TOS.

[11] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of food additives. OJ L 226, 25.6.2004, pp. 3–21.
[12] Technical dossier/p. 58 and Annex I-5.
[13] Technical dossier/pp. 58–65 and Annex I-6.
[14] Technical dossier/Annex I-7 and Additional data June 2022.
[15] Technical dossier/pp. 48–49.
[16] Technical dossier/p. 49.
[17] Technical dossier/p. 46 and Additional data June 2022.
[18] Technical dossier/p. 49 and Annex I-2.
[19] Technical dossier/pp. 50–52.
[20] Technical dossier/pp. 51–52.
[21] Technical dossier/Additional data June 2022/Annex 6.

**Table 1:** Composition of the food enzyme

| Parameters | Unit | Batches | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| **Pectinesterase activity** | PEU/g[(a)] | 153,000 | 135,000 | 140,000 |
| **Protein** | % | 22.4 | 20.9 | 19.9 |
| **Ash** | % | 0.7 | 1.2 | 1.2 |
| **Water** | % | 71.9 | 73.3 | 74.0 |
| **Total organic solids (TOS)[(b)]** | % | 27.4 | 25.5 | 24.8 |
| **Activity/TOS** | PEU/mg TOS | 558 | 529 | 565 |

(a): PEU: Pectin Esterase Units (see Section 3.3.1).
(b): TOS calculated as 100% – % water – % ash.

### 3.3.3. Purity

The lead content in the three commercial batches was below 1 mg/kg which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[22,23]

The food enzyme complies with the microbiological criteria for total coliforms, *Escherichia coli* and *Salmonella*, as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). No antimicrobial activity was detected in any of the tested batches.[22]

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, fumonisins B1, B2 and B3, ochratoxin A, trichothecenes and zearalenone was examined in the three food enzyme batches and all were below the limit of quantification (LoQ) of the applied methods.[24,25] Any adverse effects caused by the possible presence of other secondary metabolites was addressed by the toxicological examination of the food enzyme TOS.

The Panel considered that the information provided on the purity of the food enzyme is sufficient.

### 3.3.4. Viable cells and DNA of the production strain

The absence of viable cells of the production strain in the food enzyme was demonstrated [redacted].[26]

The absence of recombinant DNA in the food enzyme was tested [redacted] No DNA was detected [redacted].[27]

## 3.4. Toxicological data

### 3.4.1. Choice of test item

No toxicological studies were provided for the pectinesterase produced with *A. niger* PME. Instead, the applicant argued that the assessment of the pectinesterase could be based on toxicological data from another food enzyme – a xylanase produced with *A. niger* XEA, previously submitted to EFSA (Question No EFSA-Q-2015-00045) following the EFSA guidance (EFSA, 2009a).

The production strain of the xylanase was developed from the same recipient strain ([redacted]) as that of the pectinesterase under assessment, using a similar genetic modification system ([redacted]

[22] Technical dossier/Annexes: I-3 and I-4.
[23] LoD: Pb = 0.006 mg/L sample solution.
[24] Technical dossier/Annexes: I-3 and I-4 and Additional data June 2022/Annex 5.
[25] LoQs: fumonisins B1, B2 and B3 = 10 μg/kg each; ochratoxin A = 1 μg/kg; aflatoxins = 1 μg/kg; T-2 and HT-2 toxin = 1 μg/kg each; deoxynivalenol = 10 μg/kg; zearalenone = 2 μg/kg.
[26] Technical dossier/Additional data June 2022/Annex 2.
[27] Technical dossier/Spontaneous data_submission_2023-05-12/ Annex Absence of DNA in the food enzyme pectinesterase strain PME.

[redacted]), with a specific gene of interest in each case. The genetic modification in *A. niger* PME only differs from that of *A. niger* XEA in the gene of interest and in the number of expression cassettes inserted in the genome. WGS analysis confirmed that no partial sequences of the expression cassettes were inserted in the genome of the production strain. No rounds of mutagenesis have been applied in the development of the production strains from the recipient and all the genetic modifications have been described throughout and raise no concerns. Therefore, the genetic differences between *A. niger* PLA and *A. niger* XEA are not expected to result in a different toxigenic potential.

The batch of xylanase food enzyme from *A. niger* XEA used for toxicological studies was produced according to a standard procedure similar to the one described in Section 3.2 of this opinion.[21] The raw materials used and the steps involved in the manufacturing of the xylanase and pectinesterase food enzymes are comparable in both processes,[28] and the temperature and pH conditions used during fermentation are similar. Small differences in raw materials were noted, but none of these differences raised concern.

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, all made with the substitute food enzyme. Taking the microbiological and technical data into account, the Panel considered the xylanase as a suitable surrogate for the pectinesterase in the toxicological studies.

### 3.4.2. Genotoxicity

#### 3.4.2.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was performed according to the OECD Test Guideline 471 (OECD, 1997a) and following good laboratory practice (GLP).[29] Four strains of Salmonella Typhimurium (TA98, TA100, TA1535, TA1537) and *Escherichia coli* WP2*uvrA* pKM 101 were used with or without metabolic activation (S9-mix), applying the plate incorporation assay.

Two independent experiments were carried out in triplicate using five concentrations of the food enzyme ranging from 50 to 5,000 μg dry matter/plate of the food enzyme (corresponding to 49–4,932 μg TOS/plate).

No precipitation or significant cytotoxicity was observed in any strain at any concentration tested. Upon treatment with the food enzyme, there was no significant increase in the number of revertant colonies in any tester strain, either in the presence or absence of metabolic activation.

The Panel concluded that the surrogate food enzyme xylanase did not induce gene mutations in the bacterial reverse mutation assay under the test conditions employed for this study.

#### 3.4.2.2. *In vitro* mammalian chromosomal aberration test

The *in vitro* mammalian chromosomal aberration test was carried out according to the OECD Test Guideline 473 (OECD, 1997b) and following GLP.[30]

Chinese hamster ovary cells (CHO) were treated with the food enzyme in the absence and presence of metabolic activation (S9-mix). Based on the results obtained in a dose-range finding test, the cells were treated with 1,250, 2,500 and 5,000 μg dry matter/mL (corresponding to 1,233, 2,466 and 4,932 μg TOS/mL) in a short-term treatment (3 + 17 h of recovery) in the presence and absence of S9-mix, and with 750, 3,000 and 5,000 μg dry matter/mL (corresponding to 740, 2,959 and 4,932 μg TOS/mL) applying a continuous treatment (20 h without recovery) in the absence of S9-mix.

No precipitation or significant changes in pH were detected. Cytotoxicity, measured as mitotic inhibition, did not exceed 23% at any concentration of the food enzyme. No statistically significant increase in the frequency of chromosomal aberrations was observed in the short-term treated cultures compared to the negative controls either in the presence or absence of S9-mix. After continuous treatment in the absence of S9-mix, a statistically significant increase in the frequency of aberrant cells was observed only at 5,000 μg dry matter/mL (2.5% aberrant cells vs. 0% in the negative controls). However, the increase was slightly above the historical negative control range (0–2%), consistent with published control data, and consequently, it was not considered biologically relevant.

[28] Technical dossier/Additional data June 2022/Annex 7.
[29] Technical dossier/Annex I-16.
[30] Technical dossier/Annex I-17.

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

### 3.4.3. Repeated dose 90-day oral toxicity study in rodents

The repeated dose 90-day oral toxicity study in rodents was performed according to OECD test guideline 408 (OECD, 1998) and following GLP.[31] Groups of 10 male and 10 female Wistar rats received daily via gavage for at least 90 days dose levels of 400, 1,600 and 6,400 mg food enzyme/kg bw per day corresponding to 0, 116, 463 and 1,852 mg TOS/kg bw per day. Controls received the vehicle (double distilled water).

No mortality was observed.

In the functional observations, a statistically significantly increase in the landing foot splay in mid- and high-dose males (+20% and 35%, respectively) and in mid- and high-dose females (+28% and 30%, respectively), in the grip strength of the hind limb in mid-dose males (+12%) and in high-dose females (+15%), and in the grip strength of the fore limb in mid- and high-dose females (+14% and + 10%, respectively) were observed. Furthermore, a statistically significant decrease in the grip strength of the fore limb in low-dose males (−7%) was observed. The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (decrease in the grip strength of the fore limb), there was no dose–response relationship (decrease in the grip strength of the fore limb in males, increase in the grip strength of the hind limb in males, increase in the fore limb grip strength in females) and there were no changes in other relevant parameters (e.g. in gait, motor activity or righting reflex).

Haematological investigations revealed a statistically significant increase in mean corpuscular haemoglobin (MCH) in mid-dose males (+5%), in mean corpuscular haemoglobin concentration (MCHC) in mid- and high-dose males (+4% at both dose levels), in platelet count (Plat, + 13%), prothrombin time (PT, +11%), neutrophil percentage (Neu%, +80%) all in mid-dose males, a decrease in lymphocyte percentage (Lymph%, −14%) in mid-dose males, an increase in haematocrit (HCT) in high-dose females (+5%) and an increase in mean corpuscular volume (MCV) in mid- and high-dose females (+4% at both dose levels). The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (all parameters), there was no dose–response relationship (MCH, MCHC, Plat, PT, Neu% and Lymph% in males, MCV), the changes were not accompanied by changes in total number of white blood cells (Neu% and Lymph% in males and females), the changes were small (HCT, MCV, MCH, MCHC, Plat) and there were no changes in other related parameters (for HCT, MCV, MCH, MCHC in number of red blood cells and haemoglobin concentration).

Clinical chemistry investigations revealed statistically significant increases in concentrations of creatinine in mid- and high-dose males (+10% and 14%, respectively), chloride (+3%) and sodium (+2%) in high-dose males. The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (all parameters), the changes were small (chloride and sodium) and there were no histopathological changes in the kidneys (creatinine).

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

The Panel identified a no observed adverse effect level (NOAEL) of 1,852 mg TOS/kg bw per day, the highest dose tested.

### 3.4.4. Allergenicity

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

The potential allergenicity of the pectinesterase produced with the genetically modified *A. niger* strain PME 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, two matches were found.[32] The matching allergens were Sal k 1 pectin methylesterase from Russian thistle (*Salsola kali*) and Ole e 11 pectinesterase from olive tree (*Olea europaea*), known as respiratory allergens.

[31] Technical dossier/Annex I-18.
[32] Technical dossier/pages 81–83/Annex I-19.

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 and Russian thistle pollen.

[redacted], a known source of allergen, is present in the media fed to 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 considered that no potentially allergenic residues from this source are present in the food enzyme.

The Panel considered that, under the intended conditions of use, the risk of allergic reactions upon dietary exposure to this food enzyme, particularly in individuals sensitised to pollen allergens, cannot be excluded.

## 3.5. Dietary exposure

### 3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in two 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)] |
|---|---|---|
| Fruit and vegetable processing for juice production | Fruits and vegetables mash | 0.4–**1.1** |
| Fruit and vegetable processing for products other than juices | Fruits and vegetables | 0.4–**3.6** |

(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.
(c): Technical dossier/3rd submission/ p. 70 and Additional data June 2022.

In fruit juice production, the food enzyme is added to the crushed mash, where the pectinesterase de-esterifies pectins to promote the action of other pectinases and facilitate the release of juice. The food enzyme is also added to the extracted juice during the clarification step to aid the hydrolysis of the pectic material contributing to cloudiness.[33] The food enzyme–TOS remains in the final juices.

For other processed fruit and vegetable products,[34] pectinesterase is added to the pealed or crushed fruits/vegetables to aid the degradation of the cell wall matrix, consequently softening the fruit and vegetables. The treated fruits/vegetables may be further processed by pasteurisation, cooking or blanching in the presence of calcium. This causes the pectic acid to cross-link with calcium into calcium pectate, retaining the firmness of fruits or vegetables in the finished canned products.

Based on data provided on thermostability (see Section 3.3.1), the pectinesterase is expected to be inactivated by heat in most of the fruits 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

[33] Technical dossier/3rd submission/ pp. 67–68 and 102–103.
[34] Technical dossier/3rd submission/ pp. 67–69 and 102–103.

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 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 0.095 mg TOS/kg bw per day in infants at the 95th percentile.

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

| Population group | Estimated exposure (mg TOS/kg body weight per day) | | | | | |
|---|---|---|---|---|---|---|
| | **Infants** | **Toddlers** | **Children** | **Adolescents** | **Adults** | **The elderly** |
| **Age range** | 3–11 months | 12–35 months | 3–9 years | 10–17 years | 18–64 years | ≥ 65 years |
| **Min–max mean** (number of surveys) | 0.002–0.050 (11) | 0.008–0.048 (15) | 0.006–0.036 (19) | 0.002–0.019 (21) | 0.001–0.012 (22) | 0–0.010 (22) |
| **Min–max 95th** (number of surveys) | 0.011–0.095 (9) | 0.033–0.080 (13) | 0.020–0.074 (19) | 0.006–0.047 (20) | 0.006–0.034 (22) | 0.002–0.026 (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 | + |
| 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 overestimation of the exposure.

### 3.6. Margin of exposure

A comparison of the NOAEL (1,852 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0–0.05 mg TOS/kg bw per day at the mean and from 0.002–0.095 mg TOS/kg bw per day at the 95th percentile resulted in a margin of exposure of at least 19,495.

## 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 niger* strain PME 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 its recombinant DNA.

## 5. Documentation as provided to EFSA

Application for authorisation of pectinesterase from a genetically modified strain of *Aspergillus niger* (strain PME). January 2015. Submitted by DSM Food Specialties.

Additional information. June 2022. Submitted by DSM Food Specialties.

Spontaneous data submission. May 2020. Submitted by DSM Food Specialties.

Spontaneous data submission. May 2023. Submitted by DSM Food Specialties.

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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 |
| kDa | kiloDalton |
| LoD | limit of detection |
| LoQ | Limit of quantification |
| MoE | margin of exposure |
| OECD | Organisation for Economic Cooperation and Development |
| PCR | polymerase chain reaction |
| TOS | total organic solids |
| WHO | World Health Organization |
| WGS | whole genome sequence |

# 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.2023.8152#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).