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SCIENTIFIC OPINION

Safety evaluation of the food enzyme endo-polygalacturonase from the non-genetically modified *Aspergillus tubingensis* strain MUCL 55013

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Abstract

The food enzyme endo-polygalacturonase ((1→4)-α-D-galacturonan glycanohydrolase (endo-cleaving); EC 3.2.1.15)) is produced with the non-genetically modified *Aspergillus tubingensis* strain MUCL 55013 by Soufflet Biotechnologies. The food enzyme is free from viable cells of the production organism. It is intended to be used in 10 food manufacturing processes: processing of fruits and vegetables for the production of juices, other fruit and vegetable products, wine, distilled spirits from wine, alcoholic beverages other than grape wine; processing of plant-derived products for the production of refined and unrefined sugar, edible oils from plants, green coffee beans by demucilation, coffee extracts and tea and other herbal and fruit infusions. Since residual amounts of total organic solids (TOS) are removed in three processes, dietary exposure was calculated only for the remaining seven food manufacturing processes. Exposure was estimated to be up to 7.834 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not indicate a safety concern. The systemic toxicity was assessed by a repeated dose 90-day oral toxicity study in rats. The Panel identified a no observed adverse effect level of 2,097 mg TOS/kg bw per day, the highest dose tested, resulting in a margin of exposure of at least 268. A search for the similarity of the amino acid sequence of the food enzyme to known allergens found 14 matches, one of which was to a food allergen. The Panel considered that the risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded, in particular for individuals sensitised to papaya, but that the risk will not exceed that of consumption of papaya. In addition, oral allergy reactions cannot be excluded in pollen-sensitised individuals. Based on the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns, under the intended conditions of use.

KEYWORDS

(1→4)-α-D-galacturonan glycanohydrolase, *Aspergillus tubingensis*, EC 3.2.1.15, endo-polygalacturonase, food enzyme, non-genetically modified microorganism

CONTENTS

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[1] on food enzymes came into force. This Regulation applies to enzymes that are added to food to perform a technological function in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food, including enzymes used as processing aids. Regulation (EC) No 1331/2008[2] established the European Union (EU) procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

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

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

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

1.1 | Background and terms of reference as provided by the requestor

1.1.1 | Background as provided by the European Commission

Only food enzymes included in the Union 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-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 Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008[2], the Commission has verified that the five applications fail within the scope of the food enzyme Regulation and contain all the elements required under Chapter II of that Regulation.

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-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[1] on food enzymes.

[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.

1.2 | 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 *Aspergillus 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.

EFSA-Q-2015-00039 specifically concerns the request for EFSA to perform a scientific risk assessment on the food enzyme: Polygalacturonase from *Aspergillus niger*.

Recent data identified the production microorganism as *Aspergillus tubingensis* (Section 3.1). Therefore, this name will be used in this opinion instead of *Aspergillus niger*.

This data package, identified as EFSA-Q-2022-00283, concerns the food enzyme endo-polygalacturonase produced with *A. tubingensis* strain MUCL 55013 and submitted by Soufflet Biotechnologies.

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme polygalacturonase from a non-genetically modified *Aspergillus tubingensis* (strain MUCL 55013). The dossier was updated on 19 April 2022.

Additional information was requested from the applicant during the assessment process on 12 October 2022 and received 12 April 2023 (see 'Documentation provided to EFSA').

2.2 | Methodologies

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

A data package originated from a joint dossier should fulfil the data requirements in the 'Submission of a Dossier on Food Enzymes for Safety Evaluation' (EFSA CEF Panel, 2009). During the evaluation, the Panel applied, whenever possible, the updated current 'Scientific Guidance for the submission of dossiers on Food Enzymes' (EFSA CEP Panel et al., 2021) and the guidance on the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes '(EFSA CEP Panel et al., 2023).

3 | ASSESSMENT[6]

IUBMB nomenclature	Endo-polygalacturonase
Systematic name	(1→4)-α-D-galacturonan glycanohydrolase
Synonyms	Pectinase, pectin hydrolase, endo-D-galacturonase
IUBMB No	EC 3.2.1.15
CAS No	9032-75-1
EINECS No	232–885-6

Endo-polygalacturonases catalyse the random hydrolysis of 1,4-α-D-glycosidic linkages between galacturonic acid residues in polygalacturonans resulting in their progressive depolymerisation.

The enzyme under assessment is intended to be used in 10 food manufacturing processes: processing of fruits and vegetables for the production of juices; other fruit and vegetable products; wine; distilled spirits from wine; alcoholic

[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.

[6]Technical dossier/p. 15–16, 109–112.

beverages other than grape wine; processing of plant-derived products for the production of refined and unrefined sugar; edible oils from plants; green coffee beans by demucilation; coffee extracts; tea and other herbal and fruit infusions.

3.1 | Source of the food enzyme[7]

The endo-polygalacturonase is produced with the non-genetically modified filamentous fungus *Aspergillus tubingensis* strain MUCL 55013, which is deposited [redacted] with the deposit number [redacted].[8] The production strain was identified as *A. tubingensis* by [redacted].[9]

A search for genes involved in the biosynthesis of secondary metabolites revealed that the production strain may have the ability to synthesise TAN-1612, a polyketide anhydrotetracycline with a potential neuropeptide Y antagonistic activity. TAN-1612 itself does not have the tetracycline antibiotic pharmacophores and therefore does not have antibiotic activity. The absence of its antimicrobial activity against Gram-positive and negative bacteria and yeasts was verified by Baldera-Aguayo et al. (2022).

3.2 | Production of the food enzyme[10]

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 [redacted].[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[15]

The endo-polygalacturonase activity derives from two isozymes, endo-polygalacturonase I and II. Both are single polypeptide chains of [redacted] and [redacted] amino acids, respectively.[16] The molecular masses of the mature proteins, calculated from their amino acid sequences, are [redacted] kDa (endo-polygalacturonase I) and [redacted] kDa (endo-polygalacturonase II).[16] The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.[17] A consistent protein pattern was observed across all batches. The gel showed a protein band corresponding to an apparent molecular mass of about 37 kDa, consistent with the expected mass of the two isozymes.[18] The food enzyme was found to contain pectin esterase, pectin lyase and cinnamoyl esterase activities.[19] No other enzyme activities were reported.

The in-house determination of polygalacturonase activity[20] is based on the hydrolysis of polygalacturonic acid (reaction conditions: pH 4.9, 30°C) by measuring the release of reducing groups using 2-cyanoacetamide spectrophotometrically at 280 nm. The enzyme activity is expressed in polygalacturonase units/g (U/g). One unit is defined as the amount of enzyme that liberates 1 µmol of reducing groups per min under the conditions of the assay.[20]

[7]Technical dossier/p. 38–48, 112–120; Technical dossier/Additional data, 12 April 2023.
[8]Technical dossier/Annex 14.
[9]Technical dossier/Annex 15.
[10]Technical dossier/p. 49–63; Technical dossier/Annex 16; Annex 18.
[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/Annex 16.
[13]Technical dossier/Annex 18–1; Technical dossier/p. 50/Figure 10.
[14]Technical dossier/p. 51–55/Table 15; Table 16.
[15]Technical dossier/p. 16–17; 23–27; Technical dossier/Additional data, 12 April 2023.
[16]Technical dossier/p. 20.
[17]Technical dossier/p. 21–23; Technical dossier/Annex 2.
[18]Technical dossier/p. 21–23; Technical dossier/p. 22/Figure 2; Technical dossier/Annex 2.
[19]Technical dossier/p. 26; Technical dossier/Annex 4.
[20]Technical dossier/Annex 3.

The food enzyme has a temperature optimum around 50°C (pH 4.9) and a pH optimum around pH 5 (30°C). Thermostability was determined by pre-incubation for 10 min at various temperatures. The endo-polygalacturonase activity decreased above 55°C and was fully inactivated by pre-incubation at 65°C.[21]

3.3.2 | Chemical parameters[22]

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (Table 1), one of which was used for the toxicological studies. The mean total organic solids (TOS) of the three food enzyme batches was 14.6% and the mean enzyme activity/TOS ratio was 131.9 U/mg TOS.

TABLE 1 Composition of the food enzyme.[d]

		Batches		
Parameters	**Unit**	**1**	**2**	**3**[a]
Polygalacturonase activity	U/g[b]	18,048	18,988	20,640
Protein	%	12.3	12.2	10.6
Ash	%	0.53	0.55	0.65
Water	%	84.5	84.6	85.3
Total organic solids (TOS)[c]	%	15	14.9	14
Activity/TOS ratio	U/mg TOS	120.3	127.0	147.3

[a]Batch used for the toxicological studies.
[b]U: Polygalacturonase Unit (see Section 3.3.1).
[c]TOS calculated as 100% – % water – % ash.
[d]Technical dossier/p. 28–29; 95–96; Technical dossier/Annex 5.

3.3.3 | Purity[23]

The lead content[24] in all batches was below 5 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). In addition, arsenic and mercury contents were below the limits of quantification (LoQ) of the employed methods.[25,26] For cadmium, the average concentration was 0.03 mg/kg. The Panel considered this concentration as not of concern.

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).[27] No antimicrobial activity was detected in any of the tested batches.[28]

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 aflatoxin B1, B2, G1, G2, deoxynivalenol, fumonisin B1, B2, ochratoxin A, HT2-toxin, T-2 toxin and zearalenone was examined in all food enzyme batches and all were below the LoQ of the applied methods.[29,30] TAN-1612, an anhydrotetracycline, was not detected in the food enzyme using an enzyme-linked immunosorbent assay (ELISA) kit with a limit of detection level of 5 µg/L.[31] Adverse effects caused by the possible presence of other secondary metabolites were addressed by the toxicological examination of the food enzyme TOS.

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

3.3.4 | Viable cells of the production strain[32]

The absence of viable cells of the production strain in the food enzyme was demonstrated in three independent batches analysed in triplicate. For each sample, 10 mL of product was diluted in 90 mL NaCl solution. From these, 10 mL was filtered; the

[21]Technical dossier/p. 27.
[22]Technical dossier/p. 28–29; 95–96; Technical dossier/Annex 5; Annex 6; Annex 7.
[23]Technical dossier/Annex 8; Annex 9; Annex 10; Annex 11; Annex 12; Technical dossier/Additional data, 12 April 2023.
[24]Technical dossier/p. 33; Technical dossier/Additional data, 12 April 2023: Limit of detection (LoD): Pb = 0.01 mg/kg.
[25]Technical dossier/p. 33; LoQs: As = 0.01 mg/kg; Hg = 0.01 mg/kg.
[26]Technical dossier/Annex 8.
[27]Technical dossier/p. 33–34; Technical dossier/Annex 9–2, Annex 9–4, Annex 9–6; Technical dossier/Additional data, 12 April 2023.
[28]Technical dossier/Annex 17.
[29]Technical dossier/p. 34–36; LoQs: aflatoxin B1 = 1 µg/kg; aflatoxins B2, G1 and G2 = 5 µg/kg each; ochratoxin A = 2.5 µg/kg; deoxynivalenol = 100 µg/kg; fumonisin B1 and B2 = 250 µg/kg each; HT2-toxin = 10 µg/kg; T-2 toxin = 10 µg/kg; zearalenone = 6 µg/kg.
[30]Technical dossier/Annex 11.
[31]Technical dossier/Additional data, 12 April 2023/Appendix 1.
[32]Technical dossier/p. 37–38; Technical dossier/Annex 13.

filters were placed on non-selective agar plates and incubated at 30°C for 4 days. No colonies were produced. A positive control was included.[33]

3.4 | Toxicological data[34]

A battery of toxicological tests including a bacterial reverse mutation test (Ames test), an *in vitro* mammalian cell micronucleus test and a repeated dose 90-day oral toxicity study in rats has been provided. Batch 3 (Table 1) used in these studies was one of the batches intended for commercialisation and was considered a suitable test item.

3.4.1 | Genotoxicity

3.4.1.1 | *Bacterial reverse mutation test*

A bacterial reverse mutation test (Ames test) was performed according to the Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 2020) and following good laboratory practice (GLP).[35]

Five strains of *Salmonella* Typhimurium (TA98, TA100, TA102, TA1535 and TA1537) were used with or without metabolic activation (S9-mix). Two experiments were carried out in triplicate.

In the first experiment, the plate incorporation method was applied, using seven concentrations of the food enzyme of 5, 16, 50, 160, 500, 1,600 and 5,000 µg TOS/plate. Toxic effects, evident as a reduction in the number of revertant colonies or of the background lawn, occurred in *S.* Typhimurium TA100, TA1535, TA1537 and TA102 without S9-mix at 500 µg TOS/plate and above.

In the second experiment, seven concentrations of the food enzyme (80, 160, 300, 625, 1,250, 2,500 and 5,000 µg TOS/plate) were tested without S9-mix using the plate incorporation method and six concentrations (160, 300, 625, 1,250, 2500 and 5,000 µg TOS/plate) were tested with S9-mix using the preincubation method. Toxic effects, evident as a reduction of background lawn, occurred in *S.* Typhimurium TA1535 without S9-mix at 1,250 µg TOS/plate and above.

Upon treatment with the food enzyme, there was no biologically relevant increase in the number of revertant colonies above the control values in any strain tested, with or without S9-mix.

The Panel concluded that the food enzyme endo-polygalacturonase did not induce gene mutations under the test conditions applied in this study.

3.4.1.2 | *In vitro mammalian cell micronucleus test*

The *in vitro* mammalian cell micronucleus test was carried out according to the OECD Test Guideline 487 (OECD, 2016) and following GLP.[36]

Duplicate cultures of human peripheral whole blood lymphocytes were treated with the food enzyme with or without metabolic activation (S9-mix). The cells were exposed to the food enzyme and scored for the frequency of bi-nucleated cells with micronuclei (MNBN) at concentrations of 250, 1,000, 4,000 and 5,000 µg TOS/mL in a short-term treatment (3-h exposure and 21-h recovery period) without S9-mix, at concentrations of 2,500, 4,000 and 5,000 µg TOS/mL in a short-term treatment (3-h exposure and 21-h recovery period) with S9-mix and at concentrations of 500, 1,000 and 2,500 µg TOS/mL in a long-term treatment (21-h exposure without recovery period) without S9-mix. In an additional experiment, cells were exposed to the food enzyme and scored for the frequency of MNBN at concentrations of 4,000, 4,500 and 5,000 µg TOS/mL in a short-term treatment (3-h exposure and 21-h recovery period) with S9-mix.

In the short-term treatment, a cytotoxicity (based on the reduction of replication index) of 52% was observed at 5,000 µg TOS/mL without S9-mix. In the long-term treatment, a cytotoxicity of 47% was reported at 2,500 µg TOS/mL without S9-mix. The frequency of MNBN was statistically significantly different to the negative controls at a single concentration (1,000 µg TOS/mL) in the short-term treatment without S9-mix, but the value was within the historical control range and no concentration–response relationship was observed. In the short-term treatment with S9-mix, the frequency of MNBN was statistically significantly different to the negative controls at concentrations of 4,000 and 5,000 µg TOS/mL. However, this increase was not reproducible in the additional experiment and, therefore, was not considered to be of biological relevance.

The Panel concluded that the food enzyme endo-polygalacturonase did not induce an increase in the frequency of MNBN under the test conditions applied in this study.

3.4.2 | Repeated dose 90-day oral toxicity study in rodents

The repeated dose 90-day oral toxicity study followed the OECD Test Guideline 408 (OECD, 2018) and GLP[37] with the following deviations: urea was not determined, the prostate gland was not weighed and the skeletal muscle was not examined microscopically. The Panel considered that these deviations are minor and do not impact on the evaluation of the study.

[33]Technical dossier/Annex 13.
[34]Technical dossier/p. 82–96.
[35]Technical dossier/Annex 20–3153-831.
[36]Technical dossier/Annex 21–3154-368.
[37]Technical dossier/Annex 23-1-A4053.

Groups of 10 male and 10 female Hsd:Sprague–Dawley SD rats received by gavage the food enzyme in doses of 524, 1,049 or 2,097 mg TOS/kg body weight (bw) per day. Controls received the vehicle (water produced by reverse osmosis).

One high-dose female was found dead on day 9 of administration. The Panel considered the death as due to mis-dosing based on the reported macroscopic and microscopic findings.

The body weight gain was statistically significantly increased on day 8 of administration in high-dose females (+53%). The Panel considered the change as not toxicological relevant, as it was only recorded sporadically, it was only observed in one sex and the change was without a statistically significant effect on the final body weight gain.

The feed consumption was statistically significantly increased on day 57 of administration in low-dose females (+8%) and decreased on day 92 of administration in high-dose males (–3%) and on day 50 of administration in low- and high-dose females (–13%, –16%, respectively). The Panel considered the changes as not toxicologically relevant, as they were only recorded sporadically, there was no dose–response relationship (females), there was no consistency between the changes at the two different time points (females) and there was no statistically significant change in the final feed consumption or in the final body weight and body weight gain.

In the functional observational battery (FOB), a statistically significant decrease in the landing foot splay parameters LAN 1 (first measurement of distance between ink blots, in cm) and LAM (averaged measurement of distance between ink blots, in cm) was observed in low- and mid-dose males (LAN 1: –19%, –24%; LAM: –20%, –21%, respectively). The Panel considered the changes as not toxicologically relevant, as there were no changes in the landing foot splay parameter LAN 2 (second measurement of distance between ink blots, in cm), the changes were only observed in one sex, there was no dose–response relationship and there were no other changes in the FOB.

Haematological investigations revealed a statistically significant decrease in the eosinophil count and in the platelet count in high-dose females (–41% and –12%, respectively). The Panel considered the changes as not toxicologically relevant, as they were only observed in one sex (both parameters), the changes were small (both parameters) and there were no changes in other relevant parameters (for eosinophils – in other white blood cell parameters; for platelet count – in prothrombin time).

Clinical chemistry investigations revealed a statistically significant increase in alkaline phosphatase (ALP) levels in mid-dose males (+28%) and high-dose females (+38%), in the bile acid concentration in mid- and high-dose males (+106%, +156%, respectively) and in all treated female groups (+230%, +204%, +363%, respectively), in the total bilirubin level in treated female groups (45%, +39%, +49%, respectively) and in glucose in high-dose females (+26%), and a decrease in creatinine in low- and high-dose males (–12%, –18%, respectively) and in all treated female groups (–12%, –16%, –22%, respectively) and in chloride in all treated male groups (–1.2%, –1.9%, –1.6%, respectively) and in low-dose females (–1.6%). The Panel considered the changes as not toxicologically relevant, as they were only observed in one sex (bilirubin, glucose), there was no dose–response relationship (ALP in males, bile acids in females, bilirubin, creatinine in males), the changes were small (ALP, chloride) and there were no histopathological changes in liver and kidneys.

Statistically significant decrease in thyroid stimulating hormone in mid- and high-dose males (–33%, –45%, respectively) was noted. The Panel considered the changes as not toxicological relevant, as they were only observed in one sex, there were no changes in the thyroid hormones (tri-iodothyronine and thyroxine) and there were no changes in the thyroid weight and no histopathological changes in the thyroid.

The urinalysis revealed a statistically significant decrease in the urine volume in low-, mid- and high-dose males (–23%, –26%, –29%, respectively). The Panel considered the changes as not toxicologically relevant, as they were only observed in one sex, there were no changes in other relevant parameters and there were no histopathological changes in the kidneys.

Statistically significant changes in organ weights observed were an increase in the absolute and relative kidney weights in mid- and high-dose males (absolute: +15%, +12%; relative: +18%, +12%, respectively) and in the relative liver weight in mid-dose males (+7%), and a decrease in the absolute adrenal gland weight in low-dose females (–14%), in the absolute spleen weight in mid- and high-dose females (–14%, –20%, respectively), in the absolute thymus weight in mid- and high-dose females (–20%, –19%, respectively) and in the relative spleen weight in high-dose females (–16%). The Panel considered the changes as not toxicologically relevant, as they were only recorded in one sex (all organ weight changes), there was no dose–response relationship (kidney, liver, adrenal gland, thymus), the changes were small (liver, adrenal gland) and there were no histopathological changes in the kidneys, liver, spleen, adrenal glands or thymus in both sexes.

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

The Panel identified a no observed adverse effect level (NOAEL) of 2,097 mg TOS/kg bw per day, the highest dose tested.

3.4.3 | Allergenicity[38]

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

The potential allergenicity of the endo-polygalacturonase produced with the non-genetically modified *A. tubingensis* strain MUCL 55013 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, 14 matches were found.

[38]Technical dossier/p. 96–99; Technical dossier/Additional data, 12 April 2023.

One match found, *Cari p 1*/ripening-induced polygalacturonase 2 from papaya (*Carica papaya*), is known food allergen.[39] The other 13 are pollen allergens: *Zea m 13* from maize (*Zea mays*), *Sor h 13.01* (group 13 allergen) and *Sor h 13.02* (group 13 allergen) from Johnson grass (*Sorghum halepense*); *Phl p 13* from Timothy grass (*Phleum pratense*); *Pas n 13*/group 13 grass pollen allergen from Bahia grass (*Paspalum notatum*); LLP-PG from Easter lily (*Lilium longiflorum*); *Ole e 14.01* from Common olive (*Olea europaea*); *Cha o 2* from Japanese Cypress (*Chamaecyparis obtusa*); *Cry j 2* from Japanese cedar (*Cryptomeria japonica*); *Jun a 2*/pollen major allergen 2 protein from Mountain cedar (*Juniperus ashei*); *Pla a 2* from London plane (*Platanus x acerifolia*); *Pla a 2* from Oriental plane tree (*Platanus orientalis*); *Sal k 6.01* from Prickly saltwort (*Salsola kali*).

No information was available on oral and respiratory sensitisation or elicitation reactions of this enzyme.

Papaya is a source of both food and respiratory allergens (Sarkar et al., 2018; Bhowmik et al., 2021). Allergens present in papaya are chitinase, protease (papain), lysozyme and lipid transfer proteins; recently a polygalacturonase (Cari p 1) was identified. Cari p 1 cross-reacts with the same protein in papaya pollen (Poncet et al., 2020). Several studies reported occupational rhinitis and asthma in workers of industries where papain is handled (Baur & Fruhmann, 1979; Baur et al., 1982; Niinimaki et al., 1993; Soto-Mera et al., 2000; Van Kampen et al., 2005). In other studies, allergy to papaya-derived products unrelated to occupational exposure has also been described. García-Ortega et al. (1991) showed that administration of chymopapain for chemonucleolysis resulted in sensitisation in some patients. Mansfield and Bowers (1983) reported severe systemic allergic reactions mediated by papain-specific IgE in some individuals that ingested papain-containing meat tenderiser. Sensitisation to papaya does not typically occur from eating papaya fruit. However, once sensitised, individuals may suffer allergic reactions following any type of exposure to papaya or papaya-derived products (Morton, 1987). Sensitisation to papaya has been regularly found in people with oral allergy syndrome (OAS) and/or in latex-allergic patients (Isola et al., 2003). However, reports of clinically relevant allergic reactions are scarce (Mandal et al., 2009; Sharda et al., 2010; Vlaicu et al., 2011; Wan & Chiu, 2012; Bedolla-Barajas et al., 2014; Dey et al., 2014; Giangrieco et al., 2023).

The Panel noted that OAS is associated with sensitisation to many pollen allergens, including those from Japanese cedar (Bonds et al., 2019; Midoro-Horiuti et al., 2003), timothy grass (Ibarrola et al., 2004; Chiang et al., 2006) and olives (Palomares et al., 2008; Unsel et al., 2009). However, inflammation is usually restricted to the buccal cavity, since the allergens are rapidly degraded by gastric enzymes upon ingestion and seldomly leads to anaphylaxis (Sarkar et al., 2018).

The Panel considered that, under the intended conditions of use, the risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded, in particular for individuals sensitised to papaya, but that the risk will not exceed that of consumption of papaya. In addition, oral allergy reactions cannot be excluded in pollen-sensitised individuals.

3.5 | Dietary exposure

3.5.1 | Intended use of the food enzyme

The food enzyme is intended to be used in 10 food manufacturing processes at the recommended use levels summarised in Table 2.

TABLE 2 Intended uses and recommended use levels of the food enzyme as provided by the applicant.[c]

Food manufacturing process[a]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[b]
Processing of fruits and vegetables		
• Production of juices	Fruit and vegetables	0.1–**179**
	Mango and lemon pulps	100–300
• Production of fruit and vegetable products other than juices	Fruit and vegetables	1.3–**40**
• Production of wine	Grapes	0.5–**110**
• Production of distilled spirits from wine	Grapes	0.5–110
• Production of alcoholic beverages other than grape wine	Fruit	4–**10**
Processing of plant-derived products		
• Production of refined and unrefined sugar	Sugar beet, sugar cane	100–**150**
• Production of edible oils from plant	Oilseeds, olive	12–60
• Production of green coffee beans by demucilation	Coffee cherry	10–80
• Production of coffee extracts	Coffee bean	10–**80**
• Production of tea and other herbal and fruit infusions	Tea leaves	0.5–**200**

[a]The names have been harmonised by EFSA in accordance with the 'Food manufacturing processes and technical data used in enzyme exposure assessment' (EFSA CEP Panel et al., 2023).

[b]The numbers in bold were used for calculation.

[c]Technical dossier/p. 79; Technical dossier/Additional data, 12 April 2023.

[39]Technical dossier/Additional data, 12 April 2023/Appendix 2.

The food enzyme is used to treat all types of plant materials. The action of endo-polygalacturonase is to degrade galacturonans in the cell wall, thus increasing the yield of the plant products and facilitating the release of colour or flavouring compounds.

For the production of juices and other fruit and vegetable products, the food enzyme is added to fruit or vegetables during cutting or crushing to degrade pectin.[40] The food enzyme-TOS remains in the final foods.

During the production of juices, fruit and vegetable concentrates is also obtained by condensation as a flavouring preparation.[41] The applicant did not detect amino nitrogen in the flavour condensate.[42,43] The Panel considered the technical information and experimental data sufficient to demonstrate the removal of the food enzyme-TOS from these types of flavouring preparations obtained by distillation from fruit and vegetables. These flavouring preparations are usually incorporated into food products, such as reconstituted juices, purees and jams.

In wine production, the food enzyme is added to grapes during crushing and maceration.[44] The food enzyme-TOS remains in the wine.

For distilled spirits from wine, such as cognac, the food enzyme-TOS is not carried over due to distillation (EFSA CEP Panel et al., 2023).

In the manufacturing of fruit-derived alcoholic beverages, such as cider and poiré, the food enzyme is added to fruit, such as apples and pears, during maceration and to the pressed juice during clarification and before fermentation.[45] The food enzyme-TOS remains in the relevant alcoholic beverages.

In sugar production, the food enzyme is added to sugar cane during crushing and to the raw juices during depectinisation.[46] The food enzyme-TOS is not carried into the refined sugar due to the crystallisation, but remains in the unrefined molasses (EFSA CEP Panel et al., 2023).

For edible plant oil, the food enzyme is used to treat olives or oilseeds before pressing.[46] The degradation of pectin leads to higher yield of the crude oil.

For olive oils, the term 'olive oil' is defined in the Regulation (EU) No 1308/2013[47] as '*composed of refined olive oils and virgin olive oils*'. The term 'virgin olive oils' means '*oils obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions that do not lead to alterations in the oil, which have not undergone any treatment other than washing, decantation, centrifugation or filtration, to the exclusion of oils obtained using solvents or using adjuvants having a chemical or biochemical action, or by re-esterification process and any mixture with oils of other kinds*.'

In accordance with the law, the use of enzymes is not permitted in the production of virgin olive oils in the European Union. Therefore, this assessment is limited to the use of this food enzyme in the production of refined olive oil only. The food enzyme-TOS is removed from the refined olive oil in the refinement process (EFSA CEP Panel et al., 2023).

In coffee processing, the food enzyme is added firstly to the coffee cherry during fermentation to remove the mucilaginous coat. After the separation, the remaining residual food enzyme-TOS is removed from the green coffee bean by repeated washing (EFSA CEP Panel et al., 2023). In the second step, the food enzyme is used to treat the roasted coffee bean, reducing viscosity of the coffee extracts.[46] The food enzyme-TOS remains in these extracts.

For tea and herbal infusion products, polygalacturonases break down the pectin in the cell walls of tea leaves, reducing the foaming property and releasing phenolic compounds into the tea extracts.[48] The food enzyme-TOS remains in the final products.

Based on data provided on thermostability (see Section 3.3.1), the food enzyme is expected to be inactivated during most of the food manufacturing processes shown in Table 2, but may remain active in wine and juices, depending on the pasteurisation conditions.

3.5.2 | Dietary exposure estimation

In accordance with the guidance document (EFSA CEP Panel et al., 2021), a dietary exposure was calculated only for seven food manufacturing processes where the food enzyme-TOS remains in the final foods: production of juices, production of fruit and vegetable products other than juices, production of wine, production of alcoholic beverages other than grape wine, production of refined and unrefined sugar, production of coffee extracts, production of tea and other herbal and fruit infusions.

Chronic exposure to the food enzyme-TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSA CEP Panel et al., 2021). The estimation involved selection of relevant food categories

[40]Technical dossier/p. 73/Figure 12.

[41]Technical dossier/Additional data, 12 April 2023/Response 6 and Figure 6.

[42]Technical dossier/Additional data, 12 April 2023/Response 6 and Table 5.

[43]Technical dossier/Additional data, 12 April 2023/Appendix 3, LoD = 5 mg amine/l.

[44]Technical dossier/p. 75/Figure 13.

[45]Technical dossier/p. 71/Figure 11.

[46]Technical dossier/p. 76.

[47]Regulation (EU) No 1308/2013 of the European Parliament and of the Council of 17 December 2013 establishing a common organisation of the markets in agricultural products and repealing Council Regulations (EEC) No 922/72, (EEC) No 234/79, (EC) No 1037/2001 and (EC) No 1234/2007. OJ L 347, 20.12.2013, p. 671–854.

[48]Technical dossier/p. 77.

and application of technical conversion factors (EFSA CEP Panel et al., 2023). 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 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 48 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 26 European countries. The highest dietary exposure was estimated to be 7.834 mg TOS/kg bw per day in children at the 95th percentile.

TABLE 3 Summary of the estimated dietary exposure to food enzyme–TOS in six population groups.

	Estimated exposure (mg TOS/kg body weight per day)					
Population group	**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)	0.104–1.331 (12)	0.433–4.512 (15)	0.507–2.856 (19)	0.164–1.433 (21)	0.156–0.997 (22)	0.136–0.713 (23)
Min–max 95th percentile (number of surveys)	0.359–4.581 (11)	2.125–7.320 (14)	1.970–7.834 (19)	0.721–5.061 (20)	0.608–3.575 (22)	0.535–2.553 (22)

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 always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
For juice production, 179 mg TOS/kg fruit and vegetables was used for the calculation instead of the maximum proposed use level of 300 mg TOS/kg for mango and lemon pulp, as these fruit juices are minor contributors to the overall juice consumption	+/–
For the 'production of wine', the input data included both wine and wine vinegar	+
Minor FoodEx categories found to only sporadically contain molasses were excluded from the exposure assessment	–
'Brown sugar' produced through use of cane molasses or caramelised sugar syrup was excluded, due to it being a niche product on the European market	–
The transfer of food enzyme-TOS into cane and beet molasses/syrups was assumed to be 100%	+
No distinction was made between beet molasses and cane syrups used as ingredients in foods	+/–
Use of recipe fractions to disaggregate FoodEx categories	+/–
Use of technical factors in the exposure model	+/–
Exclusion of three processes from the exposure estimation: – Production of distilled spirits from wine – Production of edible oils from plant – Production of green coffee beans by demucilation	–

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

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

The exclusion of three food manufacturing processes from the exposure estimation was based on > 99% of TOS removal. This was not expected to impact on the overall estimate derived.

3.6 | Margin of exposure

A comparison of the NOAEL (2,097 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates of 0.104–4.512 mg TOS/kg bw per day at the mean and from 0.359 to 7.834 mg TOS/kg bw per day at the 95th percentile resulted in margin of exposure (MoE) of at least 268.

4 | CONCLUSIONS

Based on the data provided and the derived margin of exposure for the food manufacturing processes in which TOS was not removed from the final product, the Panel concluded that the food enzyme endo-polygalacturonase produced with the non-genetically modified *Aspergillus tubingensis* strain MUCL 55013 does not give rise to safety concerns under the intended conditions of use.

5 | DOCUMENTATION AS PROVIDED TO EFSA

Technical dossier 'Polygalacturonase' produced by a non-genetically strain of *Aspergillus tubingensis* under Regulation (EC) No 1332/2009 on food enzymes. Submitted by Soufflet Biotechnologies. The dossier was updated on 19 April 2022.

Additional information. 12 April 2023. Submitted by Soufflet Biotechnologies.

ABBREVIATIONS

ALP	alkaline phosphatase
AMFEP	Association of Manufacturers and Formulators of Enzyme Products
[redacted]	[redacted]
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
ELISA	enzyme-linked immunosorbent assay
FAO	Food and Agricultural Organisation of the United Nations
FOB	battery of functional observations
FoodEx	a standardised food classification and description system
GLP	Good Laboratory Practice
GM	genetically modified
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
MNBN	bi-nucleated cells with micronuclei
MoE	margin of exposure
NOAEL	no observed adverse effect level
non-GM	non-genetically modified
OAS	oral allergy syndrome
OECD	Organisation for Economic Cooperation and Development
RM	raw material
TOS	total organic solids
U	unit
[redacted]	[redacted]
WHO	World Health Organization

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CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

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LEGAL NOTICE

The full opinion will be published in accordance with Article 12 of Regulation (EC) No 1331/2008 once the decision on confidentiality will be received from the European Commission.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX A

Dietary exposure estimates to the food enzyme–TOS in details

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

Table 1: Average and 95th percentile exposure to the food enzyme–TOS per age class, country and survey.
Table 2: Contribution of food categories to the dietary exposure to the food enzyme–TOS per age class, country and survey.

APPENDIX B

Population groups considered for the exposure assessment

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From 12 weeks on up to and including 11 months of age	Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia, Spain
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Netherlands, Portugal, Republic of North Macedonia[a], Serbia[a], 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, Republic of North Macedonia[a], Serbia[a], Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Bosnia and Herzegovina[a], Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Montenegro[a], Netherlands, Portugal, Romania, Serbia[a], Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Bosnia and Herzegovina[a], Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro[a], Netherlands, Portugal, Romania, Serbia[a], Slovenia, Spain, Sweden
The elderly[b]	From 65 years of age and older	Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro[a], Netherlands, Portugal, Romania, Serbia[a], Slovenia, Spain, Sweden

[a] Consumption data from these pre-accession countries are included for testing purpose.

[b] 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).