SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 19 May 2022

doi: 10.2903/j.efsa.2022.7373

Safety evaluation of the food enzyme endo-1,4-β-xylanase from the genetically modified *Trichoderma reesei* strain NZYM-ER

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

Abstract

The food enzyme endo-1,4-β-xylanase (4-β-D-xylan xylanohydrolase; EC 3.2.1.8) is produced with the genetically modified *Trichoderma reesei* strain NZYM-ER by Novozymes A/S. The genetic modifications do not give rise to safety concerns. The food enzyme is considered free from viable cells of the production organism and its DNA. The food enzyme is intended to be used in brewing processes, distilled alcohol production, grain treatment for the production of starch and gluten fractions and for palm oil production. Since residual amounts of total organic solids (TOS) are removed by distillation, in palm oil production and in grain treatment for the production of starch and gluten fraction, dietary exposure was only calculated for brewing processes. Dietary exposure to the food enzyme TOS was estimated to be up to 0.09 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not indicate a safety concern. The systemic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rats. The Panel identified a no observed adverse effect level of 1,051 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 11,400. A search for similarity of the amino acid sequence of the food enzyme to known allergens was made and no match was found. The Panel considered that, under the intended conditions of use (other than distilled alcohol production) the risk of allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but the likelihood for this to occur is considered to be low. Based on the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

Keywords: endo-1,4-β-xylanase, 4-β-D-xylan xylanohydrolase, EC 3.2.1.8, *Trichoderma reesei*, genetically modified microorganism

Requestor: European Commission

Question number: EFSA-Q-2021-00140

Correspondence: fip@efsa.europa.eu

Panel members: José Manuel Barat Baviera, Claudia Bolognesi, Andrew Chesson, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Claude Lambré, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Vittorio Silano (until 21 December 2020†), Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis and Holger Zorn.

Note: 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.

Declarations of interest: The declarations of interest of all scientific experts active in EFSA's work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

Acknowledgments: The Panel wishes to thank the following for the support provided to this scientific output: Jaime Aguilera, Erik Boinowitz, Ana Gomes, Simone Lunardi, Sandra Rainieri, Helen Regina.

Suggested citation: EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré C, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mengelers M, Mortensen A, Rivière G, Steffensen I-L, Tlustos C, Van Loveren H, Vernis L, Zorn H, Glandorf B, Herman L, Andryszkiewicz M, Arcella D, Kovalkovičová N, Liu Y and Chesson A, 2022. Scientific Opinion on the safety evaluation of the food enzyme endo-1,4-β-xylanase from the genetically modified *Trichoderma reesei* strain NZYM-ER. EFSA Journal 2022;20(6):7373, 17 pp. https://doi.org/10.2903/j.efsa.2022.7373

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.

† Deceased.

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

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

Before January 2009, food enzymes other than those used as food additives were not regulated or were regulated as processing aids under the legislation of the Member States. On 20 January 2009, Regulation (EC) No 1332/2008[1] on food enzymes came into force. This Regulation applies to enzymes that are added to food to perform a technological function in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food, including enzymes used as processing aids. Regulation (EC) No 1331/2008[2] established the European Union (EU) procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

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

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

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

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

1.1.1. Background as provided by the European Commission

Only food enzymes included in the European Union (EU) Community list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7(2) of Regulation (EC) No 1332/2008[1] on food enzymes.

An application has been introduced by the applicant 'Novozymes A/S' for the authorisation of the food enzyme xylanase from a genetically modified strain of *Trichoderma reesei* (strain NZYM-ER).

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 application falls within the scope of the food enzyme Regulation and contains all the elements required under Chapter II of that Regulation.

1.1.2. Terms of Reference

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002[4], the European Commission requests the European Food Safety Authority to carry out the safety assessment on the following food enzyme: xylanase from a genetically modified strain of *Trichoderma reesei* (strain NZYM-ER), in

[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, p. 15–24.

[4] Regulation (EC) No 178/2002 Article 29 (1) of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, pp. 1–44.

accordance with Regulation (EC) No 1331/2008[2] establishing a common authorisation procedure for food additives, food enzymes and food flavourings.

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 endo-1,4-β-xylanase from the genetically modified *T. reesei* (strain NZYM-ER).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme xylanase from a genetically modified *T. reesei* (strain NZYM-ER). The dossier was updated on 27 May 2021.

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

2.2. Methodologies

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

The current 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA CEF Panel, 2009) as well as the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) have been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance to the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021a).

3. Assessment[5]

IUBMB nomenclature	Endo-1,4-β-xylanase
Systematic name	4-β-D-xylan xylanohydrolase
Synonyms	Endo-(1→4)-β-xylan 4-xylanohydrolase; xylanase; β-1,4-xylanase; β-xylanase
IUBMB No	EC 3.2.1.8
CAS No	9025-57-4
EINECS No	232-800-2

Endo-1,4-β-xylanases catalyse the random hydrolysis of 1,4-β-D-xylose linkages in xylans (including arabinoxylans), resulting in the generation of (1→4)-β-D-xylan oligosaccharides. The food enzyme is intended to be used in brewing processes, distilled alcohol production, grain treatment for the production of starch and gluten fractions and for palm oil production.[6]

3.1. Source of the food enzyme[7]

The endo-1,4-β-xylanase is produced with the genetically modified filamentous fungus *T. reesei* strain NZYM-ER, which is deposited [redacted] with deposit number [redacted].[8] The production strain NZYM-ER was identified as *T. reesei* [redacted].[9]

[5] Technical dossier/2nd submission/p. 7–11, 32, 36, 53, 55, 72.
[6] Technical dossier/2nd submission/p. 13–15, 17–19, 54–55.
[7] Technical dossier/Additional information, 13.1.2022.
[8] Technical dossier/2nd submission/Annex A2.
[9] Technical dossier/Additional information, 13.1.2022/Annex A1 Version 2.

3.1.1. Characteristics of the parental and recipient microorganisms

The parental strain is [redacted]

The recipient strain [redacted][10]

[redacted][11]

3.1.2. Characteristics of introduced sequences

[redacted][12]

[redacted][13]

3.1.3. Description of the genetic modification process

[redacted][14]

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 *T. reesei* NZYM-ER differs from the recipient strain in its capacity to produce the endo-1,4-β-xylanase enzyme [redacted][15]

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

3.2. Production of the food enzyme[16]

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[17], with food safety procedures based on hazard analysis and critical control points, and in accordance with current good manufacturing practice.[18]

The production strain is grown as a pure culture using a typical industrial medium in [redacted] with conventional process controls in place. After completion of the fermentation, [redacted]

[10] Technical dossier/2nd submission/GMM Main dossier NZYM-ER Version 2/Section 1.1.2/p. 7.
[11] Technical dossier/2nd submission/Annex A1.
[12] Technical dossier/2nd submission/Section 1.3.2./p. 12–14, 22.
[13] Technical dossier/2nd submission/GMM Main dossier NZYM-ER Version 2/Section 1.3.1./p. 11–13, and Annex C1.
[14] Technical dossier/2nd submission/Annex C2.
[15] Technical dossier/2nd submission/Annex D2.
[16] Technical dossier/2nd submission/p. 46–52; Technical dossier/Version 2/Annex 5, Annex 6.
[17] 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.
[18] Technical dossier/2nd submission/p. 12.

[redacted][19] The applicant provided information on the identity of the substances used to control the fermentation and the subsequent downstream processing of the food enzyme.[20]

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[7]

3.3.1. Properties of the food enzyme[21]

The endo-1,4-β-xylanase is a single polypeptide chain of [redacted] amino acids.[22] The molecular mass of the mature protein, calculated from the amino acid sequence, is [redacted] kDa.[23] The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE).[24] A consistent protein pattern was observed across all batches. The gels showed two major protein bands with apparent molecular masses of about 66 and 55 kDa. The protein profile also included bands of lesser staining intensity.[25] The food enzyme was tested for α-amylase, amyloglucosidase, cellulase, β-glucanase, lipase and protease activities. Only cellulase and β-glucanase activities were detected.[26] No other enzymatic activities were reported.[27]

The in-house determination of endo-1,4-β-xylanase activity is based on hydrolysis of wheat arabinoxylan (reaction conditions: pH 6.0, 50°C, 5 min). Enzyme activity is determined by measuring the release of reducing carbohydrates spectrophotometrically at 405 nm after treatment with 4-hydroxybenzoic acid hydrazide and a bismuth salt. Xylanase activity is quantified relative to an internal enzyme standard and expressed in Fungal Xylanase Units/g (FXU(S)/g).[26]

The food enzyme has a temperature optimum around 70°C (pH 4.0) and a pH optimum around pH 4.0 (37°C).[27] Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures (pH 4.0). Endo-1,4-β-xylanase activity was stable up to 70°C but decreased at higher temperatures. No residual activity was detected above 80°C.[28]

3.3.2. Chemical parameters[29]

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation and the batch produced for the toxicological tests (Table 1).[30] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation is 10.5% and the mean enzyme activity/mg TOS ratio is 14.9 FXU(S)/mg TOS.

Table 1: Composition of the food enzyme

Parameters	Unit	Batches			
		1	2	3	4[(a)]
Endo-1,4-β-xylanase activity	FXU(S)/g batch[(b)]	1,510	1,540	1,600	1,390
Protein	%	8.5	8.5	9.1	7.5
Ash	%	0.3	0.3	0.3	0.5

[19] Technical dossier/2nd submission/p. 46–52.
[20] Technical dossier/2nd submission/Annex 6.
[21] Technical dossier/2nd submission/p. 36–39; Technical dossier/2nd submission/Annex 9.
[22] Technical dossier/2nd submission/p. 32; Technical dossier/2nd submission/Annex 1.
[23] Technical dossier/2nd submission/p. 34.
[24] Technical dossier/2nd submission/p. 11, 38–39.
[25] Technical dossier/2nd submission/p. 39; Technical dossier/Version 2/Annex 3.
[26] Technical dossier/2nd submission/p. 37; Technical dossier/Version 2/Annex 3.01.
[27] Technical dossier/2nd submission/p. 11, 37–38, 74; Technical dossier/Version 2/Annex 9.
[28] Technical dossier/2nd submission/p. 37–38, 74–75; Technical dossier/Version 2/Annex 9.
[29] Technical dossier/2nd submission/p. 33, 60; Technical dossier/2nd submission/Annex 2.01, Annex 2.02, Annex 2.03; Annex 11.
[30] Technical dossier/2nd submission/p. 33; Technical dossier/Version 2/Annex 11.

Parameters	Unit	Batches			
		1	2	3	4[(a)]
Water	%	88.9	88.8	90.0	89.4
Total organic solids (TOS)[(c)]	%	10.8	10.9	9.7	10.1
Activity/mg TOS	FXU(S)/mg TOS	14.0	14.1	16.5	13.8

(a): Batch used for the toxicological studies.
(b): FXU(S): Fungal Xylanase Units (see Section 3.3.1).
(c): TOS calculated as 100% – % water – % ash.

3.3.3. Purity[31]

The lead content[32] in the three commercial batches and in the batch used for toxicological studies 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).[33] In addition, the levels of arsenic, cadmium and mercury were below the limits of detection of the employed methodologies.[34,35]

The food enzyme complies with the microbiological criteria (for total coliforms, *E. coli* and *Salmonella*) as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[36] No antimicrobial activity was detected in any of the tested batches.[37]

Strains of *Trichoderma,* in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Frisvad et al., 2018). This issue is addressed by the toxicological examination of the food enzyme TOS.[38]

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[39]

The absence of viable cells of the production strain in the food enzyme was demonstrated in three independent batches analysed in triplicate. [redacted] No colonies were produced.[40]

The absence of recombinant DNA in the food enzyme was demonstrated by [redacted] of three batches in triplicate. No DNA was detected [redacted] with a limit of detection of 10 ng spiked DNA/g food enzyme.[41]

3.4. Toxicological data[42]

A battery of toxicological tests including a bacterial gene mutation assay (Ames test), an *in vitro* micronucleus study, and a repeated dose 90-day oral toxicity study in rats has been provided. The batch 4 (Table 1) used in these studies has similar protein pattern and purity as the batches used for commercialisation, and, therefore, is considered suitable as a test item.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was performed according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997) and following Good

[31] Technical dossier/2nd submission/p. 11, 34, 60, 72; Technical dossier/2nd submission/Annex 2.04, Annex 2.06, Annex 2.07, Annex 2.08, Annex 2.09, Annex 2.10; Technical dossier/Additional information, 13.1.2022.
[32] Technical dossier/2nd submission/p. 11, 35, 60; Technical dossier/2nd submission /Annex 2.04.
[33] Technical dossier/2nd submission/p. 11, 35, 60; Technical dossier/2nd submission/Annex 11.
[34] Technical dossier/2nd submission/p. 35/LODs: Pb = 0.5 mg/kg; As = 0.3 mg/kg; Cd and Hg =.05 mg/kg.
[35] Technical dossier/2nd submission/p. 35–36, 60; Technical dossier/2nd submission/Annex 11.
[36] Technical dossier/2nd submission/p. 11, 35–36; Technical dossier/2nd submission/Annex 11.
[37] Technical dossier/2nd submission/p. 11, 35, 60; Technical dossier/2nd submission/Annex 11, Annex 2.06.
[38] Technical dossier/2nd submission/p. 11, 35; Technical dossier/2nd submission/Annex 2.07, Annex 2.08, Annex 2.09, Annex 2.10.
[39] Technical dossier/Additional information, 13.1.2022/Annex E2, Version 2.
[40] Technical dossier/2nd submission/Annex E1.
[41] Technical dosssier/Additional information, 13 January 2022/Annex E2, Version 2.
[42] Technical dossier/2nd submission/p. 16, 59–65; Technical dossier/2nd submission/Annex 7.01, Annex 7.02, Annex 7.03.

Laboratory Practice (GLP).[43] Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *E. coli* WP2uvrA(pKM101) were used with or without metabolic activation (S9-mix), applying the 'treat and plate' assay. Two separate experiments were carried out using six concentrations of the food enzyme (16, 50, 160, 500, 1,600 and 5,000 μg TOS/plate in the first experiment and 160, 300, 625, 1,250, 2,500 and 5,000 μg TOS/plate in the second experiment). No cytotoxicity was observed at any concentration of the test substance. Upon treatment with the food enzyme, there was no biologically relevant increase in the number of revertant colonies above the control values in any strain tested, with or without S9-mix.

The Panel concluded that the food enzyme endo-1,4-β-xylanase did not induce gene mutations under the test conditions applied in this study.

3.4.1.2. *In vitro* mammalian cell micronucleus assay

The *in vitro* mammalian cell micronucleus test was carried out according to OECD Guideline 487 (OECD, 2014) and following GLP.[44] A single experiment was performed with duplicate cultures of human peripheral whole blood lymphocytes. The food enzyme was tested at 3,000, 4,000 and 5,000 μg TOS/mL. The cell cultures were treated with or without metabolic activation (S9-mix) for 3 h and harvested 24 h after the beginning of treatment (3 + 21 h recovery time). Additionally, a continuous 24-h treatment without S9-mix was included with harvesting 24 h after removal of the test substance (24 + 24 h recovery time). No cytotoxicity was seen either in the short-term with and/or without S9-mix or in the long-term treatment. The frequency of bi-nucleated cells with micronuclei (MNBN) was not statistically significantly different to the negative controls at all concentrations tested.

The Panel concluded that the food enzyme endo-1,4-β-xylanase did not induce an increase in the frequency of MNBN in cultured human peripheral blood lymphocytes under the test conditions applied in this study.

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

The repeated dose 90-day oral toxicity study was performed in accordance with OECD Test Guideline 408 (OECD, 1998) and following GLP.[45] Groups of 10 male and 10 female Han Wistar rats (RccHanTM:WIST) received by gavage the food enzyme in doses of 105, 347 and 1,051 mg TOS/kg body weight (bw) per day. Controls received the vehicle (reverse osmosis water).

In week 8, one low-dose male was killed for welfare reasons after an injury of the right tibiotarsal joint.

In the functional observations, a statistically significant decrease in motor activity in week 12 (total low beam score) was observed in high-dose males (−24%). The Panel considered the change as not toxicologically relevant as it was only observed in one sex and the mean value for this group was within the historical control values.

The body weight gains were increased in high-dose males (+ 7%) and in mid- and high-dose females (+ 12% and + 4.4%) during the 13-week treatment period. The Panel considered the changes as not toxicologically relevant as the magnitude of the change was low and there was no dose–response relationship in females.

Haematological investigation revealed a statistically significant, dose-dependent decrease in haematocrit (Hct) in low-, mid- and high-dose females (−2.8, −3.5, −6.9%), a decrease in haemoglobin (Hb) concentration in high-dose females (−5.2%), a decrease in red blood cells (RBC) in mid- and high-dose females (−3.6, −6.5%), an increase in mean cell haemoglobin concentration (MCHC) (+ 2%), a decrease in white blood cell (WBC) count (−26%) and in lymphocyte (L) count (−29%) and in basophile (B) count (0.00×10^9/L vs. 0.01×10^9/L in the control) in high-dose females. In males, a statistically significant decrease in RBC (−3.6%) at mid-dose and a decrease in large unstained cells (LUC) at high-dose (−50%) were reported. In addition, reductions of activated partial thromboplastin times (APTT) at all doses and in both sexes (in low-, mid- and high-dose males: −6.3, −9.3, −8.3% and in low-, mid- and high-dose females: −7.4, −6.9, −7.4%) and increases of prothrombin times (PT) in low-, mid- and high-dose females (+ 5, + 4, + 1.4%) were recorded. The Panel considered the changes as not toxicologically relevant as the magnitude of the changes was small (RBC, Hct, Hb, MCHC, B, APTT, PT), they were only observed in one sex (Hct, Hb, MCHC, WBC,

[43] Technical dossier/2nd submission/Annex 7.01.
[44] Technical dossier/2nd submission/Annex 7.02.
[45] Technical dossier/2nd submission/Annex 7.03.

L, B, LUC, PT), there was no dose-response relationship (RBC in males, APTT, PT) and the changes were within the historical control values.

Clinical chemistry investigation revealed a statistically significant increase in plasma urea concentration in high-dose males (+ 5.3%) and an increase in creatinine concentration in mid- and high-dose females (+ 13, + 8%). The Panel considered the changes as not toxicologically relevant as the magnitude of the changes was small (urea), there was no dose-response relationship (creatinine) or as they were only observed in one sex (both parameters) and were not associated with macroscopic or histopathological changes in the kidneys.

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

The Panel identified the no observed adverse effect level (NOAEL) of 1,051 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity[46]

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-1,4-β-xylanase produced with the genetically modified *T. reesei* strain NZYM-ER was assessed by comparing its amino acid sequence with those of known allergens according to the `Scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms´ (EFSA GMO Panel, 2010). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, no match was found.[47]

No information is available on oral and respiratory sensitisation or elicitation reactions of this endo-1,4-β-xylanase.

Respiratory allergy, e.g. baker's asthma, following occupational exposure to xylanase has been described in some epidemiological studies (Elms et al., 2003; Martel et al., 2010) and case reports (Tarvainen et al., 1991; Baur et al., 1998; Merget et al., 2001). However, several studies have shown that adults with occupational asthma may be able to ingest respiratory allergens without acquiring clinical symptoms of food allergy (Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). In addition, no allergic reactions upon dietary exposure to any xylanase have been reported in the literature. Therefore, it can be concluded that an allergic reaction upon oral ingestion of endo-1,4-β-xylanase produced with the genetically modified *T. reesei* strain NZYM-ER in individuals respiratory sensitised to xylanase cannot be excluded, but the likelihood of such a reaction to occur is considered to be low.

The Panel considered that the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme can be excluded in the case of distilled alcohol production, but cannot be excluded for other uses, although the likelihood of such reactions to occur is considered to be low.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in four 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[7]

Food manufacturing process[(a)]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[(b,c)]
Brewing processes	Cereals (malted or not)	2.3–**20.1**
Distilled alcohol production processes	Cereals	4.4–40.3
Grain treatment for the production of starch and gluten fractions	Cereals (grains or grist)	4.4–40.3

[46] Technical dossier/2nd submission/p. 17, 65–67; Technical dossier/2nd submission/Annex 1, Annex 8; Technical dossier/Additional information, 13.1.2022.

[47] Technical dossier/2nd submission/p. 65–67; Technical dossier/2nd submission/Annex 8.

Food manufacturing process[(a)]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[(b,c)]
Fruit and vegetable processing for palm oil production	Palm fruits	3.4–30.2

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): Based on 14.9 FXU(S)/mg TOS.
(c): The number in bold was used for calculation.

In brewing processes, the food enzyme is added to cereals during mashing or other materials (corn, rice or sorghum) during mashing or cooking.[48] The endo-1,4-β-xylanase degrades xylan from the raw material into partially hydrolysed xylans, which reduces viscosity of the mixture and facilitate subsequent filtration steps. The food enzyme–TOS remains in beer.

The food enzyme remains in the wort. Based on data provided on thermostability (see Section 3.3.1), it is expected that the endo-1,4-β-xylanase will be inactivated during brewing processes.

In distilled alcohol production,[49] the food enzyme is added to the milled grain during slurry mixing to reduce viscosity. It may also be applied during liquefaction and fermentation. The food enzyme–TOS is not carried over with the distilled alcohols (EFSA CEP Panel, 2021b).

In grain treatment for the production of starch and gluten fractions,[50] the endo-1,4-β-xylanase is added to grain during the initial steps, such as conditioning, homogenisation and dough preparation. The degradation of the highly branched arabinoxylans reduces viscosity in the slurry and increases the yield. The food enzyme–TOS is removed in the final processed foods by repeated washing and purification steps applied during grain treatment (EFSA CEP Panel, 2021b).

In palm oil production,[51] the endo-1,4-β-xylanase is added to palm fruits to break down the hemicelluloses in the mesocarp.[7] This increases cell wall rupture during digestion, easing oil release during pressing. The crude oil is separated from solid waste and the water phase containing the food enzyme. Based on moisture content and the hydrophilic property of enzyme, the applicant estimated that > 99% of hydrophilic components including the enzyme can be removed already from crude palm oil, further removal is achieved in downstream refinery process.[52] [redacted][53] The Panel considered this information sufficient to demonstrate the complete removal of the food enzyme TOS in the palm oil.

3.5.2. Dietary exposure estimation

In accordance with the guidance document (EFSA CEP Panel, 2021a), a dietary exposure was calculated only for food manufacturing processes where the food enzyme-TOS remains in the final foods, namely brewing processes.

Chronic exposure to the food enzyme–TOS was calculated by using the maximum recommended use level (Table 2) and the FEIM-brewing calculator.[54] 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 40 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 23 European countries (Appendix B). The highest dietary exposure to the food enzyme–TOS was estimated to be about 0.09 mg TOS/kg bw per day in adults.

[48] Technical dossier/2nd submission/p. 75–78.
[49] Technical dossier/2nd submission/p. 78–79.
[50] Technical dossier/2nd submission/p. 81–82.
[51] Technical dossier/2nd submission/p. 79–81.
[52] Technical dossier/2nd submission/p. 80–81.
[53] Technical dossier/2nd submission/Annex 10.
[54] https://zenodo.org/record/4382037#.X-rMgthKjD4

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)	0–0.002 (12)	0–0.004 (16)	0–0.003 (19)	0–0.004 (20)	0.002–0.020 (22)	0.000–0.010 (21)
Min–max 95th percentile (number of surveys)	0–0 (10)	0–0.024 (14)	0–0.023 (19)	0–0.018 (19)	0.011–0.092 (22)	0.003–0.042 (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	+
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	+/–
Exclusion of other processes from the exposure assessment – Distilled alcohol production – Grain treatment for the production of starch and gluten fractions – Processing for palm oil production	–

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.

The exclusion of three food manufacturing processes (distilled alcohol production, grain treatment for the production of starch and gluten fractions processes, and palm oil production) from the exposure assessment was based on > 99% of TOS removal during these processes and is not expected to have an impact on the overall estimate derived.

3.6. Margin of exposure

A comparison of the NOAEL (1,051 mg TOS/kg bw per day) from the 90-day study in rats with the derived exposure estimates of 0–0.02 mg TOS/kg bw per day at the mean and from 0–0.092 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposure (MoE) of at least 11,424.

4. Conclusions

Food enzyme TOS are removed during distilled alcohol production, palm oil production and grain treatment for the production of starch and gluten fractions. Considering this data and the derived margin of exposure for brewing processes, the Panel concluded that the food enzyme endo-1,4-β-xylanase produced with the genetically modified *Trichoderma reesei* strain NZYM-ER does not give rise to safety concerns under the intended conditions of use.

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

5. Documentation as provided to EFSA

Technical dossier "Xylanase produced by a genetically modified strain of *Trichoderma reesei* (strain NZYM-ER)", version 2. 27 May 2021. Submitted by Novozymes A/S.

Additional information. 13 January 2022. Submitted by Novozymes A/S.

Additional information on 'Grain processing/Fate of the food enzymes'. 26 April 2018 and 13 July 2018. Provided by the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) and Starch Europe. Unpublished document.

Additional information on 'Food enzyme removal during the production of cereal based distilled alcoholic beverages' and 'Food enzyme carry-over in glucose syrups'. 22 February 2017. Provided by the Association of Manufacturers and Formulators of Enzyme Products (AMFEP). Unpublished document.

References

Armentia A, Díaz-Perales A, Castrodeza J, Dueñas-Laita A, Palacin A and Fernández S, 2009. Why can patients with baker's asthma tolerate wheat flour ingestion? Is wheat pollen allergy relevant? Allergologia Et Immunopathologia, 37, 203–204. https://doi.org/10.1016/j.aller.2009.05.001

Baur X, Sander I, Posch A and Raulf-Heimsoth M, 1998. Baker's asthma due to the enzyme xylanase - a new occupational allergen. Clinical and Experimental Allergy, 28, 1591–1593.

Brisman J, 2002. Baker's asthma. Occupational and Environmental Medicine, 59, 498–502; quiz 502, 426.

EFSA (European Food Safety Authority), 2006. Opinion of the Scientific Committee related to uncertainties in dietary exposure assessment. EFSA Journal 2006;5(1):438, 54 pp. https://doi.org/10.2903/j.efsa.2007.438

EFSA (European Food Safety Authority), 2009. Guidance of the Scientific Committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: general principles. EFSA Journal 2009;7(5):1051, 22 pp. https://doi.org/10.2903/j.efsa.2009.1051

EFSA (European Food Safety Authority), 2011. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011;9(3):2097, 34 pp. https://doi.org/10.2903/j.efsa.2011.2097

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2009. Guidance of EFSA prepared by the Scientific Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. EFSA Journal 2009;7(8):1305, 26 pp. https://doi.org/10.2903/j.efsa.2009.1305

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), 2019. Statement on the characterisation of microorganisms used for the production of food enzymes. EFSA Journal 2019;17(6):5741, 13 pp. https://doi.org/10.2903/j.efsa.2019.5741

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré C, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mengelers M, Mortensen A, Rivière G, Steffensen I-L, Tlustos C, Van Loveren H, Vernis L, Zorn H, Glandorf B, Herman L, Aguilera J, Andryszkiewicz M, Gomes A, Kovalkovicova N, Liu Y, Rainieri S and Chesson A, 2021a. Scientific Guidance for the submission of dossiers on Food Enzymes. EFSA Journal 2021;19(10):6851, 37 pp. https://doi.org/10.2903/j.efsa.2021.6851

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré C, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mengelers M, Mortensen A, Rivière G, Steffensen I-L, Tlustos C, van Loveren H, Vernis L, Zorn H, Liu Y and Chesson A, 2021b. Statement on the process-specific technical data used in exposure assessment of food enzymes. EFSA Journal 2021;19(12):7010, 38 pp. https://doi.org/10.2903/j.efsa.2021.7010

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010. Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal 2010;8(7):1700, 168 pp. https://doi.org/10.2903/j.efsa.2010.1700

Elms J, Fishwick D, Walker J, Rawbone R, Jeffrey P, Griffin P, Gibson M and Curran AD, 2003. Prevalence of sensitisation to cellulase and xylanase in bakery workers. Occupational and Environmental Medicine, 60, 802–804. https://doi.org/10.1136/oem.60.10.802

FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization), 2006. General specifications and considerations for enzyme preparations used in food processing in Compendium of food additive specifications. 67th meeting. FAO JECFA Monographs, 3, 63–67. Available online: https://www.fao.org/3/a-a0675e.pdf

Frisvad JC, Møller LLH, Larsen TO, Kumar R and Arnau J, 2018. Safety of the fungal workhorses of industrial biotechnology: update on the mycotoxin and secondary metabolite potential of *Aspergillus niger, Aspergillus oryzae*, and *Trichoderma reesei*. Applied Microbiology and Biotechnology, 102, 9481–9515. https://doi.org/10.1007/s00253-018-9354-1

Martel C, Nielsen GD, Mari A, Licht TR and Poulsen LK, 2010. Bibliographic review on the potential of microorganisms, microbial products and enzymes to induce respiratory sensitization. EFSA Supporting Publication 2010;7(9):EN-75, 95 pp. https://doi.org/10.2903/sp.efsa.2010.EN-75

Merget R, Sander I, Raulf-Heimsoth M and Baur X, 2001. Baker's asthma due to xylanase and cellulase without sensitization to alpha-amylase and only weak sensitization to flour. International Archives of Allergy and Immunology, 124, 502–505.

OECD (Organisation for Economic Co-Operation and Development), 1997. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 471: Bacterial reverse mutation test. 21 July 1997. 11 pp. Available online: https://www.oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en;jsessionid=9zfgzu35paaq.x-oecd-live-01

OECD (Organisation for Economic Co-Operation and Development), 1998. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 408: Repeated dose 90-day oral toxicity study in rodents. 21 September 1998. 10 pp. Available online: https://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en

OECD (Organisation for Economic Co-Operation and Development), 2014. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 487: *In vitro* mammalian cell micronucleus test. 26 September 2014. 26 pp. Available online: https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd-tg487-2014-508.pdf

Poulsen LK, 2004. Allergy assessment of foods or ingredients derived from biotechnology, gene-modified organisms, or novel foods. Molecular Nutrition and Food Research, 48, 413–423. https://doi.org/10.1002/mnfr.200400029

Tarvainen K, Kanerva L, Tupasela O, Grenquist-Nordén B, Jolanki R, Estlander T and Keskinen H, 1991. Allergy from cellulase and xylanase enzymes. Clinical and Experimental Allergy, 21, 609–615. https://doi.org/10.1111/j.1365-2222.1991.tb00854.x

Abbreviations

AMFEP	Association of Manufacturers and Formulators of Enzyme Products
[redacted]	[redacted]
APTT	activated partial thromboplastin time
[redacted]	[redacted]
B	basophile count
[redacted]	[redacted]
bw	body weight
CAS	Chemical Abstracts Service
[redacted]	[redacted]
EFSA CEF Panel	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
EFSA CEP Panel	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EFSA GMO Panel	EFSA Panel on Genetically Modified Organisms
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
FEIM	Food Enzyme Intake Model
FoodEx	standardised food classification and description system
FXU(S)	Fungal Xylanase Units
GLP	good laboratory practice
GM	genetically modified
GMO	genetically modified organism
Hb	haemoglobin

Hct	haematocrit
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
L	lymphocyte count
LOD	limit of detection
LUC	large unstained cells
MCHC	mean cell haemoglobin concentration
MNBN	binucleated cells with micronuclei
MoE	margin of exposure
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
[redacted]	[redacted]
PT	prothrombin time
RBC	red blood cells
RM	raw material
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	total organic solids
T. reesei	*Trichoderma reesei*
WBC	white blood cell count
[redacted]	[redacted]
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.2022.7373#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, United Kingdom
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, United Kingdom
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, United Kingdom
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, United Kingdom
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, United Kingdom
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, United Kingdom

(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).