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Safety evaluation of the food enzyme β-glucanase, xylanase and cellulase from *Mycothermus thermophiloides* (strain NZYM-ST)

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Abstract

The food enzyme has three declared activities (endo-1,3(4)-β-glucanase EC 3.2.1.6, endo-1,4-β-xylanase EC 3.2.1.8 and cellulase (endo-1,4-β-D-glucanase EC 3.2.1.4)) and is produced with a non-genetically modified *Mycothermus thermophiloides* strain by Novozymes A/S. It is intended to be used in baking and brewing processes. For the two intended uses, based on the maximum use levels recommended and individual data from the EFSA Comprehensive European Food Database, dietary exposure to the food enzyme–Total Organic Solids (TOS) was estimated to be up to 0.411 mg TOS/kg body weight (bw) per day. Genotoxicity tests did not raise a safety concern. Systemic toxicity was assessed by a repeated dose 90-day oral toxicity study in rats. From this study, the Panel identified a no observed adverse effect level (NOAEL) of at least 620 mg TOS/kg bw per day, the highest dose tested. When the NOAEL is compared to the estimated dietary exposure, this results in a margin of exposure of at least 1,500. A search was made for similarity of the amino acid sequence of the declared activities with those of known allergens. Four matches were found with endo-1,3(4)-β-glucanase to known respiratory allergens, two from dust mites and two *Aspergillus fumigatus* allergens. The Panel considered that an allergic reaction upon oral ingestion of enzymes produced by *M. thermophiloides* strain NZYM-ST in individuals respiratory sensitised to these allergens cannot be excluded, but the likelihood is considered to be low. Overall, the Panel concluded that, under the intended conditions of use and based on the data provided, this food enzyme does not give rise to safety concerns.

Keywords: food enzyme, endo-1, 3(4)-β-glucanase, endo-1, 4-β-xylanase, endo-1, 4-β-D-glucanase, EC 3.2.1.6, EC 3.2.1.8, EC 3.2.1.4, *Mycothermus thermophiloides*

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1. Introduction

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

> 'Food enzyme' means a product obtained from plants, animals or micro-organisms or products thereof including a product obtained by a fermentation process using micro-organisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.
>
> 'Food enzyme preparation' means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

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

i) it does not pose a safety concern to the health of the consumer at the level of use proposed;
ii) there is a reasonable technological need;
iii) 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 arid conditions of use provided for in Article 7 (2) of Regulation (EC) No 1332/2008 on food enzymes.

Four applications have been submitted by the companies 'Novozymes A/S' and 'AB Enzymes GmbH' for the authorisation of the food enzymes Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-AV), Beta-glucanase, Xylanase and Cellulase produced by a strain of *Humicola insolens* (strain NZYM-ST), Polylacturonase from a genetically modified strain of *Trichoderma reesei* (strain RF 6197) and Pectin esterase from a genetically modified strain of *Trichoderma reesei* (strain RF 6201).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008, the Commission has verified that the four applications fall within the scope of the food enzyme Regulation and contain all the elements required under Chapter II of that Regulation.

[1] Regulation (EC) No. 1332/2008 of the European Parliament and of the Council of 16 December 2008 on Food Enzymes and Amending Council Directive 83/417/EEC, Council Regulation (EC) No. 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L 354, 31.12.2008, pp. 7–15.

[2] Regulation (EC) No. 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, pp. 1–6.

[3] Commission Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 64, 11.3.2011, p. 15–24.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out safety assessments on the food enzymes Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-AV); Beta-glucanase, Xylanase and Cellulase produced by a strain of *Humicola insolens* (strain NZYM-ST); Polygalacturonase from a genetically modified strain of *Trichoderma reesei* (strain RF 6197) and Pectin esterase from a genetically modified strain of *Trichoderma reesei* (strain RF 6201) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of food enzyme β-glucanase, xylanase and cellulase from *Humicola insolens* strain NZYM-ST (now *Mycothermus thermophiloides*).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme β-glucanase, xylanase and cellulase from *H. insolens* (strain NZYM-ST).

Additional information was sought from the applicant during the assessment process in response to a request from EFSA sent on 13 July 2017 and 25 June 2018 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 existing guidance's of EFSA Scientific Committees.

The current 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA CEF Panel, 2009) has been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance to the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016).

3. Assessment

The food enzyme contains three declared activities:

IUBMB nomenclature:	Endo-1,3(4)-β-glucanase
Systematic name:	3-(1→3;1→4)-β-D-glucan 3(4)-glucanohydrolase
Synonyms:	Endo-1,3-β-D-glucanase; β-1,3-glucanase; 1,3-(1,3;1,4)-β-D-glucan 3(4)-glucanohydrolase
IUBMB No.:	EC 3.2.1.6
CAS No.:	62213-14-3
EINECS No.:	263-462-4

The β-glucanase catalyses the hydrolysis of 1,3- or 1-4-β-glycosidic linkages in mixed-linked β-D-glucans resulting in the generation of partially hydrolysed β-D-glucans.

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

Endo-1,4-β-xylanase catalyses the random hydrolysis of 1,4-glycosidic linkages in xylans (including arabinoxylans in which the xylan chain is substituted with arabinose residues) resulting in the generation of (1→4)-β-D-xylan oligosaccharides.

IUBMB nomenclature: Cellulase
Systematic name: 4-(1,3;1,4)-β-D-glucan 4-glucanohydrolase
Synonyms: Endo-1,4-β-D-glucanase; β-1,4-glucanase; 1,4-(1,3;1,4)-β-D-glucan 4-glucanohydrolase
IUBMB No: EC 3.2.1.4
CAS No: 9012-54-8
EINECS No: 232-734-4

Endo-1,4-β-D-glucanase (cellulase) catalyses the random hydrolysis of 1-4-β-glycosidic linkages in cellulose, resulting in the generation of (1→4)-β-D-glucan oligosaccharides.

The food enzyme is intended to be used in baking and brewing processes.

3.1. Source of the food enzyme

The food enzyme is produced with a filamentous fungus *M. thermophiloides* strain NZYM-ST. The original strain was obtained by the applicant and deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Germany) as a strain of *H. insolens* with the deposit number [redacted].[4] Subsequent to this deposition the taxonomy relating to *Humicola* and related genera of the Chaetomiaceae has been extensively reviewed and revised based on molecular methods (see Wang et al., 2019). The current classification of *M. thermophiloides* now replaces the older designations *H. insolens* and *Scytalidium thermophilum*. The production strain NZYM-ST was obtained from the original strain through a selection process leading to isolates with significantly increased enzyme production. An examination of the production strain confirmed its identity as *M. thermophiloides* based on the sequence analysis of a number of characteristic regions ([redacted]) and comparison with the holotype.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No. 852/2004[5], with food safety procedures based on Hazard Analysis and Critical Control Points (HACCP), and in accordance with current Good Manufacturing Practice (GMP).

The production strain is grown as a pure culture using a typical industrial medium in a submerged, fed-batch fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration leaving a supernatant containing the food enzyme. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in which enzyme protein is retained while most of the low molecular weight material passes the filtration membrane and is discarded. 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.[6]

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

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The β-glucanase, xylanase and cellulase are single polypeptide chains of [redacted], [redacted] and [redacted] amino acids, respectively, for the entire coding sequence (i.e. including the signal peptide). The molecular masses, derived from the amino acid sequence, were calculated to be [redacted], [redacted] and [redacted] kDa, respectively.[7] The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis. A consistent protein pattern was observed across all batches. The observed complexity of the protein profiles reflects the fact that the food enzyme is derived from a wild-type fungal strain without protein fractionation.[8] The food enzyme was tested for protease,

[4] Technical dossier/p. 55-56 and Additional data December 2018.
[5] 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.
[6] Technical dossier/p. 60.
[7] Technical dossier/Additional data September 2017.
[8] Technical dossier/p.42.

α-amylase and glucoamylase activities. Only low levels of protease were detected. No other enzymatic side activities were reported.[9]

The in-house determination of β-glucanase activity is based on the random hydrolysis of the substrate β-glucan with a consequent increase in reducing groups (reaction conditions: pH 5.0, 50°C, 20 min). The reaction is stopped by adding *p*-hydroxybenzoic acid hydrazide (PAHBAH) and bismuth (III)-tartrate, which complexes with the reducing carbohydrates, producing a colour (reaction conditions: alkaline pH, 50°C, 20 min). The enzymatic activity is then determined spectrophotometrically. Enzyme activity is expressed as Fungal Beta Glucanase Units (FBG)/g. One FBG unit is defined as the amount of enzyme liberating reducing carbohydrates at a rate corresponding to 1 μmol glucose per minute under conditions specified.[10]

The xylanase and cellulase activities are quantified with the same method as the β-glucanase activity, but using different substrates and reaction conditions: wheat arabinoxylan (reaction conditions: pH 6.0, 50°C, 5 min) and carboxymethyl cellulose (reaction conditions: pH 5.0, 50°C, 20 min), respectively. The xylanase and cellulase activities are measured relative to an internal enzyme standard and expressed as Fungal Xylanase Units (FXU)/g and as Endo-Glucanase Units (EGU)/g, respectively.[11]

The optimum temperature (pH 5) is around 60°C for the β-glucanase and cellulase, and around 50°C for xylanase. At 30°C, the β-glucanase showed a broad optimum pH range between pH 3 and 8, while the activity curves show a more defined optimum of around pH 6 for the xylanase, and pH 5 for the cellulase. Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures (pH 5). All three activities decreased at temperatures greater than 50°C, with no residual activity detected at 80°C.[12]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for four food enzyme batches, three batches used for commercialisation (1, 2 and 3) and two batches (2 and 4) used for the toxicological tests (Table 1). The average Total Organic Solids (TOS) of the three food enzyme batches for commercialisation was 20.0% (range 17.7–23.1%). The average enzyme activity/TOS ratios of the three food enzyme batches for commercialisation are 1.67 FBG/mg TOS (β-glucanase), 15.60 FXU/mg TOS (xylanase) and 3.80 EGU/mg TOS (cellulase).

Table 1: Compositional data of the food enzyme[(a)]

Parameter	Unit	Batches			
		1	2[(b)]	3	4[(c)]
β-Glucanase activity	FBG/g batch[(d)]	233	364	417	45
Xylanase activity	FXU/g batch[(e)]	3,540	2,980	2,660	644
Cellulase activity	EGU/g batch[(f)]	574	897	809	82
Protein	%	13.4	13.7	14.1	3.6
Ash	%	0.8	1.1	1.3	0.6
Water	%	81.5	79.6	75.6	93.3
Total Organic Solids (TOS)[(g)]	%	17.7	19.3	23.1	6.1
β-Glucanase activity/mg TOS	FBG/mg TOS	1.32	1.89	1.81	0.74
Xylanase activity/mg TOS	FXU/mg TOS	20.0	15.4	11.5	10.56
Cellulase activity/mg TOS	EGU/mg TOS	3.24	4.65	3.50	1.34

(a): Technical dossier/p.41 and 74, and December 2018.
(b): Commercial batch also used for the bacterial reverse mutation assay.
(c): Batch used for the chromosomal aberration test and the repeated dose study.
(d): FBG/g: Fungal Beta-Glucanase units/g (see Section 3.3.1).
(e): FXU/g: Fungal Xylanase Units/g (see Section 3.3.1).
(f): EGU/g: Endo-Glucanase Units/g (see Section 3.3.1).
(g): TOS calculated as 100% - % water - % ash.

[9] Technical dossier/p. 54.
[10] Technical dossier/Annex 2.01.
[11] Technical dossier/Annexes 2.02 and 2.03.
[12] Technical dossier/Annexes 7.01, 7.02 and 7.03.

3.3.3. Purity

The lead content in the three commercial batches was below 0.5 mg/kg and in the batch used for toxicological studies (batch 4) was below 1 mg/kg which complies with the specification for lead (≤ 5 mg/kg) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006). In addition, the levels of arsenic, cadmium and mercury were below the limits of detection of the employed methodologies.[13,14]

The food enzyme complies with the microbiological criteria as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006), which stipulate that *Escherichia coli* and *Salmonella* species are absent in 25 g of sample and total coliforms should not exceed 30 colony forming units per gram.[15] No antimicrobial activity was detected in any of these batches (FAO/WHO, 2006). [13]

Strains of *Mycothermus* in common with most filamentous fungi may have the capacity to produce a range of secondary metabolites. The applicant did not provide information on the secondary metabolites produced under the conditions of fermentation which might contribute to the food enzyme TOS. The possible presence of secondary metabolites is addressed by the toxicological examination of the food enzyme TOS.

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

3.3.4. Viable cells of the production strain

The production strain was recorded as absent in the certificate of analysis of the three batches intended for commercialisation.[7]

3.4. Toxicological data

A battery of toxicological tests including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian chromosomal aberration test, and a repeated dose 90-day oral toxicity study in rats was provided. The test item for the bacterial gene mutation assay was one of the batches intended for commercialisation (Table 1, batch 2). For the remaining studies, batch 4 (Table 1) was used. This batch has a similar protein pattern as the batches used for commercialisation, but has lower chemical purity, and thus is considered suitable as a test item.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was performed according to OECD Test Guideline 471 (OECD, 1997) and following Good Laboratory Practice (GLP).[16] Four *Salmonella* Typhimurium strains (TA98, TA100, TA1535 and TA1537) and a strain of *E. coli* (WP2*uvr*A) were used in the presence or absence of metabolic activation, applying the 'treat and wash' method. Two independent experiments were carried out in triplicate using seven different concentrations of the food enzyme delivering 5, 15, 50, 150, 500, 1,500, and 5,000 μg TOS/plate. No significant cytotoxicity was observed in any strain at any dose level tested.

An increase in revertant colony numbers, which the study authors considered to have arisen from traces of histidine remaining in the food enzyme solution, was observed with strains TA98, TA100 and TA1537 in both absence and presence of S9-mix in the first test. Therefore, the first test was repeated for these three strains with two post-treatment washes and the two-washes were introduced into the protocol for the second test. No evidence of mutagenic activity was seen at any concentration of the food enzyme in the first test with strains TA1535 or WP2*uvr*A or in the repeated tests with TA98, TA100 and TA1537 or with any of the five strains in the second mutation test.

The Panel concluded that the food enzyme has no mutagenic activity under the conditions employed in this study.

[13] Technical dossier/p.41 and 74, and Additional data September 2017.
[14] Limit of detections (LODs): Commercial batches: Pb = 0.5 mg/kg; As = 0.1 mg/kg; Cd = 0.05 mg/kg; Hg = 0.03 mg/kg; Batch used for toxicological studies: Pb = 1 mg/kg; As = 0.3 mg/kg; Cd = 0.05 mg/kg; Hg = 0.05 mg/kg.
[15] Technical dossier/p. 45 and 74, and Additional data September 2017.
[16] Technical dossier/Annex 6.01 and Additional data December 2018.

3.4.1.2. *In vitro* mammalian chromosomal aberration test

The *in vitro* mammalian chromosome aberration test was carried out according to the OECD Test Guideline 473 (OECD, 1983) and following GLP.[17] The food enzyme was tested for its ability to induce chromosomal aberrations in human peripheral blood lymphocytes with and without metabolic activation (S9-mix) at concentrations up to 5,000 μg/mL (corresponding to 305 μg TOS/mL final culture concentration). Two independent experiments were performed in duplicate applying a short-term treatment in the presence of S9-mix (3 h followed by 17 h of recovery), and a continuous treatment without S9-mix (20 h). In experiment 2, the effect of a single concentration (5,000 μg/mL corresponding to 305 μg TOS/mL) was also investigated at a delayed sampling time applying a short-term treatment (3 + 41 h, with S9-mix) and a continuous treatment (44 + 0 h, without S9-mix). Based on the results of a dose-finding test, three consecutive concentrations were selected for microscopic analysis: 2,450, 3,500 and 5,000 μg/mL (corresponding to 149.5, 213.5 and 305 μg TOS/mL). Cytotoxic effects were observed at the highest concentrations (up to 24% mitotic inhibition in the presence of S9-mix in the short treatment; up to 59% mitotic inhibition in the continuous treatment experiment without metabolic activation). The enzyme preparation did not induce a significant increase in structural or numerical chromosome aberrations in cultured human blood lymphocytes, in either of the two independently repeated experiments.

The Panel concluded that the food enzyme did not induce chromosome aberrations in cultured human blood lymphocytes, under the test conditions employed for this study.

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

The repeated dose 90-day oral toxicity study was performed in accordance with OECD Test Guideline 408 (OECD, 1981), and following GLP.[18] Groups of 10 male and 10 female Wistar rats Crl:(WI)WUBR received by gavage the food enzyme in doses corresponding to 60, 200, and 620 mg TOS/kg body weight (bw) per day. Controls received the vehicle (tap water).

No mortality was observed.

The feed intake was slightly but statistically significantly lower in high-dose males in the first three weeks (differences to the control group: 4%, 5.4% and 5.2%, respectively) and statistically significantly higher in low-dose males in weeks 11 and 12 (differences to the control group of 5% and 4.5%, respectively). As the differences in feed intake did not exhibit a dose dependency, they were transitory and did not affect the growth or feed efficiency, thus they were considered not of toxicological significance.

A statistically significant decrease in urinary density was found in males from the low- and high-dose groups this finding was not accompanied by an increase in urinary volume. As the decrease in urinary density was not dose related, not associated with changes in relevant clinical chemistry parameters or in weight and morphology of the kidneys, it was considered not of toxicological significance.

No other statistically significant differences compared to controls were observed.

The Panel noted that this study was performed in 1997 and that the functional observations were not performed as these examinations were added to the OECD TG 408 under update in 1998. As the clinical condition and behaviour of all animals in the study were not affected by the treatment, and the ophthalmoscopic examination did not reveal any treatment related effects, the Panel considered that this study provided sufficient information to conclude on systemic toxicity of the food enzyme.

The Panel identified a no observed adverse effect level (NOAEL) of 620 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity

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

The potential allergenicity of the β-glucanase, xylanase and cellulase produced with the *M. thermophiloides* strain NZYM-ST was assessed by comparing its amino acid sequence with those of known allergens according to the scientific opinion on the assessment of allergenicity of genetically modified plants and microorganisms and derived food and feed of the Scientific Panel on Genetically

[17] Technical dossier/Annex 6.02.
[18] Technical dossier/Annex 6.03.

Modified Organisms (EFSA GMO Panel, 2010). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, no matches were found for xylanase and cellulase, but four matches were found for β-glucanase. Two matches were found with house dust mite (*Dermatophagoides pteronyssinus*) allergens Der p 15.0101 and Der p 15.0102 and two matches were found with the *Aspergillus fumigatus* allergens Asp f9 and Asp f16.

No information is available on oral and respiratory sensitisation or elicitation reactions of β-glucanase, xylanase or cellulase produced with the *M. thermophiloides* strain NZYM-ST.

Mite allergens (Bessot and Pauli, 2011) and *A. fumigatus* allergens (Chaudhary and Marr, 2011) are well known allergens that can trigger respiratory sensitisation and asthma. Moreover, several cases of respiratory allergy following occupational inhalation of aerosols containing xylanase and cellulase have been reported (Elms et al., 2003; Martel et al., 2010). No allergic reactions upon dietary exposure to any β-glucanase, xylanase and cellulase have been reported in the literature. Therefore, it can be concluded that the likelihood of an allergic reaction upon oral ingestion of this β-glucanase, xylanase and cellulase, produced with the *M. thermophiloides* strain NZYM-ST, in individuals respiratory sensitised to β-glucanase, xylanase and cellulase, cannot be excluded, but the likelihood of such a reaction to occur is considered to be low.

According to the information provided, substances or products that may cause allergies or intolerances (Regulation (EU) No 1169/2011[19]) are used as raw materials ([redacted]) in the media fed to the microorganisms. However, during the fermentation process, these products will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the fungal biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that potentially allergenic residues of these foods employed as protein sources are not expected to be present.

The Panel considers that under the intended condition of use the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme cannot be excluded but 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 two food processes. Intended uses and the recommended use levels are summarised in Table 2.[20]

Table 2: Intended uses and recommended use levels of the food enzyme as provided by the applicant[21]

Food manufacturing process[(a)]	Raw material	Recommended dosage of the food enzyme
Baking processes	flour	Xylanase – up to 34.5 mg TOS/kg flour (corresponding to 540 FXU/kg flour)
Brewing processes	grits	β-Glucanase – up to 14.9 mg TOS/kg grits (corresponding to 25 FBG/kg grits)
		Xylanase – up to 16.0 mg TOS/kg grits (corresponding to 250 FXU/kg grits)
		Cellulase – up to 10.5 mg TOS/kg grits (corresponding to 40 EGU/kg grits)

TOS: Total Organic Solids; FXU: Fungal Xylanase Units; FBG: Fungal Beta-Glucanase units; EGU: Endo-Glucanase Units.
(a): The description provided by the applicant has been harmonised by EFSA according to the 'EC working document describing the food processes in which food enzymes are intended to be used' - not yet published at the adoption of this opinion.

[19] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

[20] The original intended uses proposed by the applicant were: 'Brewing processes', 'Baking processes' and 'Other cereal-based processes'. In the course of the evaluation, the applicant informed EFSA about withdrawal of the intended use in 'Other cereal-based processes'.

[21] Technical dossier/p. 69 and Additional data September 2017.

In baking processes, the food enzyme is added to the raw materials during the preparation of the dough. The action of the food enzyme is primarily dependent on its xylanase content which is used to hydrolyse (arabino)xylans, present in flour which can interact with gluten and bind water. Hydrolysis of (arabino)xylans can contribute to the reduction of dough viscosity, facilitating the handling of the dough and leading to improved crumb structure and increased volume.[22]

In brewing processes, the food enzyme is added at the beginning of the mashing step. The food enzyme has the capacity to degrade the cell walls, promoting the release of starch and protein and increasing the brewing yield. The food enzyme will also aid beer filtration.

The food enzyme remains in the dough and beer. Based on data provided on thermostability (see Section 3.3.1), it is expected that the β-glucanase, xylanase and cellulase would be inactivated during baking and brewing processes.

3.5.2. Dietary exposure estimation

Chronic exposure was calculated using the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016). The assessment involved selection of relevant food categories from the EFSA Comprehensive European Food Consumption Database and application of process and technical conversion factors (Annex B in EFSA CEF Panel, 2016).

Chronic exposure was calculated by combining the maximum recommended use level provided by the applicant (see Table 2) with the relevant FoodEx categories (Annex B in EFSA CEF Panel, 2016), based on individual consumption data. Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period and normalised for bodyweight. 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 average 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 B – Tables 1 and 2. For the present assessment, food consumption data were available from 35 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix A).

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)	0.006–0.096 (10)	0.073–0.206 (14)	0.083–0.199 (19)	0.046–0.128 (18)	0.037–0.096 (19)	0.036–0.071 (18)
Min–max 95th percentile (number of surveys)	0.038–0.411 (8)	0.182–0.351 (12)	0.162–0.374 (19)	0.101–0.263 (17)	0.081–0.200 (19)	0.072–0.123 (18)

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.

[22] Technical dossier/p. 94–95.

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	+/–

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 a considerable overestimation of the exposure.

3.6. Margin of exposure

A comparison of the NOAEL (620 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates of 0.006–0.206 mg TOS/kg bw per day at the mean and from 0.038 to 0.411 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposure (MOE) of at least 1500.

4. Conclusions

Based on the data provided, and the derived margin of exposure, the Panel concluded that the food enzyme containing β-glucanase, xylanase and cellulase activities produced with *M. thermophiloides* strain NZYM-ST does not give rise to safety concerns under the intended conditions of use.

Documentation provided to EFSA

1) Technical dossier 'Beta-glucanase, xylanase and cellulase produced by a strain of *Humicola insolens* (strain NZYM-ST)'. December 2014. Submitted by Novozymes A/S.
2) Additional information, September 2017. Submitted by Novozymes A/S.
3) Additional information, December 2018. Submitted by Novozymes A/S.

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Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
CFU	colony forming units
EC	Enzyme Commission
EGU	Endo-Glucanase Units
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
FBG	Fungal Beta Glucanase Units
FXU	Fungal Xylanase Units
GLP	Good Laboratory Practice
GMO	EFSA Panel on Genetically Modified Organisms
GMP	Good Manufacturing Practices
HACCP	Hazard Analysis and Critical Control Points
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
MOE	margin of exposure
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
PAHBAH	*p*-hydroxybenzoic acid hydrazide
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	Total Organic Solids
WHO	World Health Organization

Appendix A – 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, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, United Kingdom
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Spain, United Kingdom
Children[(a)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, 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, Italy, Latvia, Netherlands, Portugal, Spain, Sweden, United Kingdom
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Spain, Sweden, United Kingdom
The elderly[(a)]	From 65 years of age and older	Austria, Belgium, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, 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).

Appendix B – 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.2019.5631).

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