SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 18 May 2022

doi: 10.2903/j.efsa.2022.7358

Safety evaluation of the food enzyme β-galactosidase from the genetically modified *Bacillus licheniformis* strain NZYM-BT

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, Magdalena Andryszkiewicz, Davide Arcella, Ana Gomes, Natalia Kovalkovicova, Yi Liu, Rita Ferreira de Sousa, Karl-Heinz Engel* and Andrew Chesson

Abstract

The food enzyme β-galactosidase (β-D-galactoside galactohydrolase; EC 3.2.1.23) is produced with the genetically modified *Bacillus licheniformis* strain NZYM-BT by Novozymes A/S. The genetic modifications do not give rise to safety concerns. The production strain has been shown to qualify for the qualified presumption of safety (QPS) status. The food enzyme was considered free from viable cells of the production organism and its DNA. It is intended to be used in milk processing for the hydrolysis of lactose. Based on the assumption that all selected milk and milk products are enzymatically treated, dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.34 mg TOS/kg body weight (bw) per day in European populations. Toxicological data were reported and were considered as supporting evidence of the safety of the food enzyme. 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 672 mg TOS/kg bw per day, the highest dose tested, which when compared with the estimated dietary exposure, results in a margin of exposure above 1,950. A search for similarity of the amino acid sequence of the food enzyme to known allergens was made and one match was found. The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, especially in individuals sensitised to galactosidase or to the matching allergen of pollen from *Platanus*. 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: food enzyme, β-galactosidase, β-D-galactoside galactohydrolase, EC 3.2.1.23, *Bacillus licheniformis*, genetically modified microorganism

Requestor: European Commission

Question number: EFSA-Q-2015-00093

Correspondence: fip@efsa.europa.eu

* Member of the Working Group on Enzymes of the EFSA Panel Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) until 3 July 2018.

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, 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: Daniele Cavanna, Giulio di Piazza, Christine Horn.

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, Andryszkiewicz M, Arcella D, Gomes A, Kovalkovicova N, Liu Y, Ferreira de Sousa R, Engel K-H and Chesson A, 2022. Scientific Opinion on the safety evaluation of the food enzyme β-galactosidase from the genetically modified *Bacillus licheniformis* strain NZYM-BT. EFSA Journal 2022;20(7):7358, 17 pp. https://doi.org/10.2903/j.efsa.2022.7358

ISSN: 1831-4732

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

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

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

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

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

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

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

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

1.1.1. Background as provided by the European Commission

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

Five applications have been introduced by the companies "Novozymes A/S", "AB Enzymes GmbH", "Ajinomoto Europe SAS" and "Nagase (Europa) GmbH" for the authorisation of the food enzymes beta-galactosidase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-BT), mannan endo-1,4-beta mannosidase (β-mannanase) from a genetically modified strain of *Trichoderma reesei* (strain RF6232), transglutaminase from *Streptoverticillium mobaraense* (strain S-8112), maltogenic amylase from a genetically modified strain of *Bacillus subtilis* (strain NZYM-SM) and glucanase from *Streptomyces violaceoruber* (strain pGlu).

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

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

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

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

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessment on the food enzymes beta-galactosidase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-BT), mannan endo-1,4-beta mannosidase (β-mannanase) from a genetically modified strain of *Trichoderma reesei* (strain RF6232), transglutaminase from *Streptoverticillium mobaraense* (strain S-8112), maltogenic amylase from a genetically modified strain of *Bacillus subtilis* (strain NZYM-SM) and glucanase from *Streptomyces violaceoruber* (strain pGlu) 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-galactosidase from a genetically modified *Bacillus licheniformis* strain (NZYM-BT).

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-galactosidase from a genetically modified *Bacillus licheniformis* (strain NZYM-BT).

Additional information was spontaneously provided by the applicant on 9 November 2020 and also requested from the applicant during the assessment process on 5 November 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, 2009b) and following the relevant guidance documents of the EFSA Scientific Committee.

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

3. Assessment

IUBMB nomenclature:	β-galactosidase
Systematic name:	β-D-galactoside galactohydrolase
Synonyms:	lactase; β-lactosidase
IUBMB No:	EC 3.2.1.23
CAS No:	9,031-11-2
EINECS No:	232–864-1

β-Galactosidases catalyse the hydrolysis of lactose to its monosaccharide units, D-glucose and D-galactose. The enzyme under this assessment is intended to be used in milk processing for the hydrolysis of lactose.

3.1. Source of the food enzyme

The β-galactosidase is produced with *B. licheniformis* strain NZYM-BT, which is deposited in the German Collection of Microorganisms and Cell Cultures (DSMZ, Germany) with the deposit number [redacted].[4] The strain was identified as *B. licheniformis* [redacted][5]

The species *B. licheniformis* is included in the list of organisms for which the qualified presumption of safety (QPS) may be applied, provided that the absence of acquired antimicrobial resistance (AMR)

[4] Technical dossier/Confidential/Annex 4 – GMM dossier/Annex A3.
[5] Technical dossier/Spontaneous additional information November 2020.

genes and toxigenic activity are verified for the specific strain used (EFSA, 2007; EFSA BIOHAZ Panel, 2020). The absence of cytotoxic activity was confirmed based on the detection of lactate dehydrogenase release from VERO cells.[5] [redacted] did not identify known genes encoding AMR.[6] Therefore, the production strain is considered to qualify for the QPS approach.

3.1.1. Characteristics of the parental and recipient microorganisms

The parental strain is [redacted]
The recipient strain, [redacted]

3.1.2. Characteristics of the introduced sequences

[redacted]

[6] Technical dossier/Spontaneous additional information November 2020 and Additional information February 2022/Annex_Answers and Appendices 1 and 2.
[7] Technical dossier/Confidential/Annex 4 – GMM dossier.

[redacted][7]

3.1.3. Description of the genetic modification process

The purpose of the genetic modification was to enable the production strain to synthesise β-galactosidase [redacted][8]

The absence of the antimicrobial resistance genes [redacted] was demonstrated [redacted][6]

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 *B. licheniformis* NZYM-BT differs from the recipient strain [redacted] in its capability to produce [redacted] β-galactosidase [redacted][6]

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

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[9], with food safety procedures based on hazard analysis and critical control points, and in accordance with current good manufacturing practice.[10]

The production strain is grown as a pure culture using a typical industrial medium in a [redacted] 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 food enzyme is then further purified and concentrated, including an ultrafiltration step in which enzyme protein is retained while most of the low molecular mass material passes the filtration membrane and is discarded.[11] 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.[12]

[8] Technical dossier/Confidential/Annex 4 – GMM dossier/Annex D1.
[9] 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.
[10] Technical dossier, pp. 46–52/Annex 5.
[11] Technical dossier, pp. 46–52.
[12] Technical dossier/Annexes: 2.06 and 6; Spontaneous additional information November 2020.

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-galactosidase is a dimer of [redacted] amino acids per subunit.[13] The molecular mass of the mature protein was calculated from the amino acid sequence to be [redacted] kDa.[13] The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. A consistent protein pattern was observed across all batches. In accordance with the calculated molecular mass, the gels showed a major protein band in all batches, migrating above the 97 kDa reference protein, together with bands of lower staining intensity below 97 kDa.[14] The food enzyme was tested for α-amylase, glucoamylase, lipase and protease activities and none were detected.[15,16]

The in-house determination of β-galactosidase activity is based on the hydrolysis of *o*-nitrophenyl-β-D-galactopyranoside (reaction conditions: pH 6.5, 30°C, 10 min). The enzymatic activity is determined by measuring the release of *o*-nitrophenol spectrophotometrically at 405 nm. The enzyme activity is quantified relative to an internal enzyme standard and expressed in lactase activity units (B-standard)/g (LAU(B)/g).[17]

The food enzyme has a temperature optimum between 40°C and 50°C (pH 6.5) and a pH optimum around pH 6.0 (37°C). Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures (pH 6.5). Enzyme activity decreased above 40°C, showing no residual activity above 60°C.[18]

3.3.2. Chemical parameters

Data on chemical parameters of the food enzyme were provided for three batches used for commercialisation and one batch produced for the toxicological tests (Table 1).[19] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation is 6.8% and the mean enzyme activity/TOS ratio is 107 LAU(B)/mg TOS.

Table 1: Composition of the food enzyme

Parameter	Unit	Batches			
		1	2	3	4[(a)]
β-Galactosidase activity	LAU(B)/g batch[(b)]	6,140	6,840	8,690	6,730
Protein	%	4.4	5.0	5.6	NA
Ash	%	0.5	0.3	0.2	2.9
Water	%	92.6	92.8	93.3	90.7
Total Organic Solids (TOS)[(c)]	%	6.9	6.9	6.5	6.4
β-Galactosidase activity/mg TOS	LAU (B)/mg TOS	89	99	134	105

NA: not analysed.
(a): Batch used for the toxicological studies.
(b): LAU (B)/g: lactase activity units (B standard)/g (see Section 3.1.3).
(c): TOS calculated as 100% – % water – % ash.

3.3.3. Purity

The lead content in the three commercial batches and in the batch used for toxicological studies was below 0.5 mg/kg[20] which complies with the specification for lead (≤ 5 mg/kg) as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). In

[13] Technical dossier, p. 31/Annex 1.
[14] Technical dossier, p. 33.
[15] LODs: alpha-amylase = 0.3 KNU(T)/g; glucoamylase = 0.825 AGU/g; lipase = 0.02 KLU/g; protease = 0.0056 AU(N)/g.
[16] Technical dossier pp. 39–40/Annexes: 3.02, 3.03, 3.04, 3.05.
[17] Technical dossier: Annex 3.01.
[18] Technical dossier pp. 38–39/Annex 9.
[19] Technical dossier pp. 32, 57–58/Annex 7.02; Spontaneous additional information November 2020.
[20] Technical dossier/Annex 7.02; Spontaneous additional information November 2020.

addition, the levels of arsenic, cadmium and mercury were below the limits of detection of the employed methodologies.[20,21]

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).[22] No antimicrobial activity was detected in any of the tested batches (FAO/WHO, 2006).[20]

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

3.3.4. Viable cells and DNA of the production strain

The absence of viable cells of the production strain in the food enzyme was demonstrated in three independent batches analysed in triplicate. [redacted] No colonies were produced. A positive control was included.[23]

The absence of recombinant DNA in the food enzyme was demonstrated by PCR analysis of three batches analysed in triplicate. No DNA was detected [redacted][24]

3.4. Toxicological data

As the production strain qualifies for the QPS approach to safety assessment and as no issue of concern arising from the production process of the food enzyme were identified (see Sections 3.1, 3.2 and 3.3), the Panel considers that no toxicological studies other than assessment of allergenicity are necessary. A battery of toxicological tests including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian cell micronucleus assay 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 chemical purity as the batches used for commercialisation, and thus is considered suitable as a test item. The tests are reported as supporting evidence of the safety of the food enzyme.

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 laboratory practice (GLP).[25] Four strains of Salmonella Typhimurium (TA1535, TA100, TA1537, TA98) and *Escherichia coli* WP2uvrA(pKM101) were used in the presence or absence of metabolic activation (S9-mix), applying the 'treat and plate assay'. Two experiments in triplicate were carried out using six concentrations of the food enzyme (156–5,000 μg dry matter/plate, corresponding to 107, 215, 430, 860, 1,720 and 3,441 μg TOS/plate). A third experiment in triplicate was carried out with *S.* Typhimurium TA1535 and TA1537 at the same concentrations in the presence of S9-mix. In *Salmonella* strains, the exposure to the highest concentrations of food enzyme caused a reduction in viable cell count, on contrary in *E. coli* WP2uvrA(pKM101) a growth stimulation was observed, probably due to an abundance of various nutrients present in lactase. Upon treatment with the food enzyme, there was no significant increase in revertant colony numbers above the control values in any strain with or without S9-mix.

The Panel concluded that the food enzyme ß-galactosidase did not induce gene mutations under the test conditions employed for this study.

3.4.1.2. *In vitro* mammalian cell micronucleus test

The *in vitro* micronucleus assay was carried out according to the OECD Test Guideline 487 (OECD, 2010) and following GLP.[26] The dose range-finding study was performed at concentrations of 18.14–5,000 μg/mL, and no inhibition of the replication index was observed. Based on these results, duplicate cultures of human peripheral blood lymphocytes were exposed to the food enzyme at 3,000,

[21] LoDs: Pb = 0.5 mg/kg; As = 0.1 mg/kg; Cd = 0.05 mg/kg; Hg = 0.05 mg/kg.
[22] Technical dossier pp. 35–36/Annex 7.02.
[23] Technical dossier/Confidential/Annex 4 – GMM dossier/Annex E1.
[24] Technical Dossier/Additional information February 2022/Annex E2 – Version 2.
[25] Technical dossier/Annex 7.01.
[26] Technical dossier/Annex 7.02.

4,000 and 5,000 μg dry matter/mL (corresponding to 2,065, 2,753 and 3,441 μg TOS/mL) in the short-term treatment (3 h followed by 21 h recovery period) with and without metabolic activation (S9-mix), and at 500, 2,000 and 5,000 μg/mL (corresponding to 344, 1,376 and 3,441 μg TOS/mL) in the continuous treatment (24 h, followed by 24 h recovery period) in the absence of S9-mix. No cytotoxicity was observed after treatments, both in the presence and absence of S9-mix. The frequency of binucleated cells with micronuclei (MNBN) was comparable to the negative controls at all concentrations tested; exception was the 3 + 21-h treatment in the absence of S9-mix at 3,000 μg/mL, where a statistically significant increase in the frequency of MNBN was observed. The Panel noted that this value was within the 95% of the historical control range, and therefore, it was considered not to be biologically relevant.

The Panel concluded that the food enzyme ß-galactosidase did not induce an increase in the frequency of MNBN in cultured human peripheral blood lymphocytes, under the test conditions employed 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 according to OECD Test Guideline 408 (OECD, 1998) and following GLP.[27] Groups of 10 male and 10 female Sprague–Dawley (Crl:CD(SD)) rats received by gavage the food enzyme in doses of 67, 222 or 672 mg TOS/kg bw per day. Controls received the vehicle (reverse osmosis water).

No mortality was observed.

In the functional observations, a statistically significant decrease in the hindlimb grip strength was observed in mid- and high-dose females (−9.8% and −15.7%) and a statistically significant decrease in motor activity for the 30-min period in high-dose males (−70.4%). The Panel considered these changes as not toxicologically relevant as they were only observed in one sex (both parameters), the change was within the laboratory historical control data range (hindlimb grip strength) and the change was only recorded sporadically (motor activity).

Haematological investigation revealed a statistically significantly increased mean cell haemoglobin (MCH) in mid- and high-dose males (+3.5% and +2.3%), mean cell haemoglobin concentration (MCHC) in all treated males (+1.8%, +1.5% and +1.8%), a decrease in reticulocyte count (−18.9%) and red cell distribution width (RDW, −7.7%) in high-dose males and an increase in large unstained cell count (LUC, +71.4%) in low-dose females. The Panel considered these changes as not toxicologically relevant as they were only observed in one sex (all parameters), the magnitude of the changes was small (MCH, MCHC, RDW), there was no dose–response relationship (MCH, MCHC, LUC), the changes were not accompanied by a change in erythrocyte count (reticulocyte count) and the changes were within the historical control values (MCH, MCHC).

Clinical chemistry investigation revealed a statistically significant increase in alkaline phosphatase (ALP) activity in mid- and high-dose males (+26% and +24%) and in high-dose females (+49%), a decrease in sodium (−0.7%), urea (−12%) and creatinine (−17%) concentrations and an increase in potassium concentrations (+16%) in high-dose males. The Panel considered the changes as not toxicologically relevant as they were only recorded in one sex (sodium, urea, creatinine), there was no dose–response relationship (ALP in males), the magnitude of the changes was low (ALP, sodium), the control values were at the low end of the historical control values (ALP), there were no changes in other biochemical markers for liver function (ALP) and there were no histopathological changes in the liver (ALP) or in the kidneys (potassium, sodium, creatinine).

There was a statistically significant decrease in adjusted brain weight in mid- and high-dose males (−4.7% and −2.9%). The Panel considered this change as not toxicologically relevant as it was only observed in one sex, there was no dose–response relationship, the magnitude of the change was low and there were no histopathological changes in the brain.

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

The panel identified the no observed adverse effect level (NOAEL) of 672 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.

[27] Technical dossier/Annex 7.03.

The potential allergenicity of the β-galactosidase produced with the genetically modified *B. licheniformis* strain NZYM-BT 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, one match was found. The matching allergen was Pla o 2 produced by the oriental plane tree (*Platanus orientalis*), a known respiratory allergen.[28]

No information is available on oral and respiratory sensitisation or elicitation reactions of this β-galactosidase.

Pla o 2 is a polygalacturonase produced by *Platanus orientalis* known as occupational respiratory allergen, with cross-reacting homologues in various grass pollen and edible plants. Sensitisation to pollen from *Platanus* trees is associated with the oral allergy syndrome (Enrique et al., 2002). In this syndrome, allergic reactions occur mainly in the mouth (Midoro-Horiuti et al., 2003). Such reactions are seldomly leading to severe systemic anaphylaxis; however, oral allergy may not be excluded after consumption.

Some studies have shown that adults with occupational asthma to an enzyme used in food can commonly ingest the corresponding allergen without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Brisman, 2002; Poulsen, 2004, Armentia et al., 2009). A few case reports are available that describe allergic reactions upon oral exposure to lactase or β-galactosidase in individuals, respiratorily sensitised to β-galactosidase (Stöcker et al., 2016; Voisin and Borici-Mazi, 2016).

According to the information provided, substances or products that may cause allergies or intolerances (Regulation (EU) No 1169/2011[29]) are used as raw materials ([redacted]). In addition, [redacted], known source of allergen, is also present in the media fed to the microorganisms. However, during the fermentation process, these products will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the microbial 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 considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme cannot be excluded, especially in individuals sensitised to galactosidase or to pollen from *Platanus*.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in milk processing for the hydrolysis of lactose at an intended use level up to 7,500 LAU(B)/kg lactose. Considering that cow's milk contains about 5% lactose, this corresponds to 3.54 mg TOS/kg milk.[30]

To hydrolyse lactose in milk, the food enzyme is added to pasteurised milk or ultra-high-temperature-treated milk. Depending on the methods applied, the enzyme-treated milk may be pasteurised again. No separation step is applied to remove the enzyme from the final foods (lactose-reduced milk and milk products).[31]

Based on data provided on thermostability (see Section 3.3.1), it is expected that the β-galactosidase is inactivated if heat treatment is applied after the addition of the food enzyme.

3.5.2. Dietary exposure estimation

Chronic exposure to the food enzyme–TOS was calculated by combining the maximum recommended use level provided by the applicant with the individual data from the EFSA Comprehensive European Food Consumption Database. The estimation involved selection of relevant

[28] Technical dossier/pp. 63–66/Annexes: 8.01 and 8.02.

[29] 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.

[30] Technical dossier / pp. 54–56; Spontaneous additional information November 2020.

[31] Technical dossier / pp. 75–77.

food categories and application of technical conversion factors (EFSA CEP Panel, 2021b). 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).

In the EU, milk and dairy products are widely consumed by consumers of all age groups. The prevalence of lactose malabsorption has been reported to range from 19% to 37% in western, southern and northern Europe (Storhaug et al., 2017). Symptoms of lactose malabsorption (i.e. lactose intolerance) are generally avoided by limiting or avoiding foods and drinks that contain lactose. Consequently, lactose-intolerant individuals, who opt to consume dairy products will choose products with very low lactose content (either naturally low or with reduced content). Lactose-reduced milk is readily available in the EU; however, other lactose-reduced products, such as soft cheese, quark and yoghurt, are available to a much lesser degree. The Comprehensive Database currently does not provide sufficient detail to estimate food intake specifically for lactose-intolerant population groups. Exposure was therefore estimated based on the assumption that lactose-intolerant people may exert similar consumption patterns of dairy products as non-lactose-intolerant population groups. However, to reflect on the relative low availability of lactose-reduced dairy products other than milk, two scenarios were calculated.

The first scenario (A) assumes that all milk and dairy products are lactose reduced due to enzyme treatment. The second scenario (B) considers all milk as lactose reduced, but assumes that only a fraction of other dairy products are lactose-reduced due to the enzymatic treatment. Scenario A is more conservative than scenario B. The selection of the relevant milk and milk products and the technical factors applied were subject to a public consultation.[32]

Table 2 provides an overview of the derived exposure estimates across all surveys. Detailed mean and 95th percentile exposure to the food enzyme–TOS per age class, country and survey, as well as contribution from each FoodEx category to the total dietary exposure are reported in Appendix A – Tables 1 and 2. For the present assessment, food consumption data were available from 41 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). Under scenario A, the highest dietary exposure to the food enzyme–TOS was estimated to be about 0.3 mg TOS/kg bw per day in young children below 3 years of age. Under scenario B, the highest dietary exposure to the food enzyme–TOS was estimated to be about 0.25 mg TOS/kg bw per day in toddlers. The shift of age group seen under these two scenarios reflects the expansion of food items by age in young children.

Table 2: 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
Scenario A – all milk and dairy products consumed are enzymatically lactose reduced products						
Min–max mean (number of surveys)	0.005–0.104 (11)	0.056–0.140 (15)	0.056–0.119 (19)	0.017–0.052 (21)	0.012–0.028 (22)	0.010–0.025 (22)
Min–max 95th (number of surveys)	0.033–0.344 (9)	0.167–0.302 (13)	0.109–0.206 (19)	0.043–0.109 (20)	0.031–0.064 (22)	0.029–0.056 (21)
Scenario B – all milk and a fraction of dairy products consumed are enzymatically lactose reduced products						
Min–max mean (number of surveys)	0.001–0.052 (11)	0.003–0.100 (15)	0.019–0.092 (19)	0.001–0.034 (21)	0.001–0.013 (22)	0.001–0.011 (22)

[32] https://www.efsa.europa.eu/en/call/call-input-data-exposure-assessment-food-enzymes-7th-call

Population group	Estimated exposure (mg TOS/kg body weight per day)					
	Infants	Toddlers	Children	Adolescents	Adults	The elderly
Min–max 95th (number of surveys)	0.004–0.166 (9)	0.067–0.251 (13)	0.054–0.163 (19)	0.002–0.072 (20)	0.003–0.041 (22)	0.011–0.029 (21)

3.5.3. Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the following sources of uncertainties have been considered and are summarised in Table 3.

Table 3: Qualitative evaluation of the influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction of impact
	Exposure to food enzyme–TOS
Model input data	
Consumption data: different methodologies/representativeness/under-reporting/ misreporting/no portion size standard	+/–
Use of data from food consumption survey of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Possible national differences in categorisation and classification of food	+/–
Model assumptions and factors	
Exposure to food enzyme–TOS was always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
Inclusion of semi-soft and soft cheeses that are ripened normally less than 3 months	+
Use of recipe fractions in disaggregation FoodEx categories likely to contain the food enzyme	+/–
Use of technical factors in the exposure model	+/–
For risk characterisation, the worst case (scenario A) was selected, which assumes that all the selected milk/dairy products are enzymatically lactose-reduced	+

+: 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 an overestimation of the exposure.

3.6. Margin of exposure

The intake derived under the exposure scenario A, the most conservative scenario, was used for the calculation of the margin of exposure.

A comparison of the NOAEL (672 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates of 0.005–0.140 mg TOS/kg bw per day at the mean and from 0.029 to 0.344 mg TOS/kg bw per day at the 95th percentile, resulted in a margin of exposure (MoE) of at least 1,953.

4. Conclusions

Based on the data provided, the outcome of the QPS assessment of the production strain and the derived margin of exposure as supporting evidence, the Panel concluded that the food enzyme β-galactosidase produced with the genetically modified *Bacillus licheniformis* strain NZYM-BT 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 provided to EFSA

1) Dossier "β-Galactosidase produced by a genetically modified strain of *Bacillus licheniformis* (strain NZYM-BT)", February 2015. Submitted by Novozymes A/S.
2) Summary report on genotoxicity and subchronic toxicity study related to β-galactosidase produced with a strain of *Bacillus licheniformis* (strain NZYM-BT) by Novozymes A/S. Delivered by FoBiG GmbH (Freiburg, Germany) on 14 March 2016.
3) Spontaneous additional information. November 2020. Submitted by Novozymes A/S.
4) Additional information. February 2022. Submitted by Novozymes A/S.

References

Armentia A, Dias-Perales A, Castrodeza J, Dueñas-Laita A, Palacin A and Fernándes 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

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

Cullinan P, Cook A, Jones M, Cannon J, Fitzgerald B and Newman Taylor AJ, 1997. Clinical responses to ingested fungal α-amylase and hemicellulase in persons sensitized to *Aspergillus fumigatus*? Allergy, 52, 346–349. https://doi.org/10.1111/j.1398-9995.1997.tb01003.x

[redacted]

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), 2007. Opinion of the Scientific Committee on a request from EFSA on the introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. EFSA Journal 2007;5(12):587, 16 pp. https://doi.org/10.2903/j.efsa.2007.587

EFSA (European Food Safety Authority), 2009a. Guidance of EFSA prepared by the Scientific Panel of Food Contact Material, 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 (European Food Safety Authority), 2009b. 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 BIOHAZ Panel (EFSA Panel on Biological Hazards), 2020. Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA (2017–2019). EFSA Journal 2020;18(2):5966, 56 pp. https://doi.org/10.2903/j.efsa.2020.5966

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, 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

FAO/WHO (Food and Agriculture Organization of the United States/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, pp. 63–67. Available online: https://www.fao.org/3/a-a0675e.pdf

Enrique E, Cisteró-Bahíma A, Bartolomé B, Alonso R, San Miguel-Moncín MM, Bartra J and Martínez A, 2002. *Platanus acerifolia* pollinosis and food allergy. Allergy, 57, 351–356. https://doi.org/10.1034/j.1398-9995.2002.1s3509.x

Midoro-Horiuti T, Mathura V, Schein CH, Braun W, Yu S, Watanabe M, Lee JC, Brooks EG and Goldblum RM, 2003. Major linear IgE epitopes of mountain cedar pollen allergen Jun a 1 map to the pectate lyase catalytic site. Molecular Immunology, 40, 555–562. https://doi.org/10.1016/s0161-5,890(03)00168-8.

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/docserver/9789264071247-en.pdf?expires=1639400502&id=id&accname=guest&checksum=D2E89C16911BE1BEB13BBC206B402098

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), 2010. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 487: In vitro mammalian cell micronucleus test. 22 July 2010. 29 pp. Available online: https://www.oecd-ilibrary.org/environment/test-no-487-in-vitro-mammalian-cell-micronucleus-test_9789264091016-en

Poulsen LK, 2004. Allergy assessment of foods or ingredients derived from biotechnology, gene-modified organisms, or novel food. Molecular Nutrition and Food Research, 48, 413–423.

Stöcker B, Grundmann S, Mosters P, Nitzsche P and Brehler R, 2016. Occupational sensitization to lactase in the dietary supplement industry. Archives of Environmental and Occupational Health, 71, 259–267.

Storhaug CL, Fosse SK and Fadnes LT, 2017 Country, regional, and global estimates for lactose malabsorption in adults: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol. 2, 738–746. https://doi.org/10.1016/S2468-1253(17)30154-1.

Voisin MR and Borici-Mazi R, 2016. Anaphylaxis to supplemental oral lactase enzyme. Allergy Asthma and Clinical Immunology, 12, 66. https://doi.org/10.1186/s13223-016-0171-8. eCollection 2016.

Abbreviations

CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
DSMZ	German Collection of Microorganisms and Cell Cultures
FAO	Food and Agriculture Organisation of the United Nations
GLP	good laboratory practice
GMO	genetically modified organisms
IUBMB	International Union of Biochemistry and Molecular Biology
LAU	lactase activity units
MNBN	bi-nucleated cells with micronuclei
MoE	margin of exposure
PCR	polymerase chain reaction
QPS	qualified presumption of safety
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TOS	total organic solids
WGS	whole genome sequencing
WHO	World Health Organisation

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.2022.7358#support-information-section).

The file contains two sheets, corresponding to two tables.

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

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

Appendix B – Population groups considered for the exposure assessment

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From 12 weeks on up to and including 11 months of age	Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Netherlands, Portugal, Slovenia, Spain
Children	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
The elderly[(a)]	From 65 years of age and older	Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden

(a): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011).