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Safety evaluation of the food enzyme α-amylase from the genetically modified *Bacillus licheniformis* strain NZYM-BC

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Abstract

The food enzyme α-amylase (4-α-D-glucan glucanhydrolase; EC 3.2.1.1) is produced with the genetically modified *Bacillus licheniformis* strain NZYM-BC by Novozymes A/S. The genetic modifications do not give rise to safety concerns. The production strain was shown to qualify for the qualified presumption of safety (QPS) status. The food enzyme was free from viable cells of the production organism and its DNA. It is intended to be used in six food manufacturing processes, namely starch processing for the production of glucose syrups and other starch hydrolysates, distilled alcohol production, brewing processes, cereal-based processes, refined and unrefined sugar production and fruit and vegetable processing for juice production. Since the residual amounts of total organic solids (TOS) are removed by distillation and by the purification steps applied during the production of glucose syrups, dietary exposure was not calculated for these two food manufacturing processes. For the remaining four processes, the dietary exposure to the food enzyme–TOS was estimated to be up to 0.05 mg TOS/kg body weight per day in European populations. Genotoxicity tests did not raise safety concern. The similarity of the amino acid sequence of the food enzyme to those of known allergens was searched 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, but the likelihood was 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: food enzyme, α-amylase, 4-α-D-glucan glucanhydrolase, EC 3.2.1.1, glycogenase, *Bacillus licheniformis*, genetically modified microorganism

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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:

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

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

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

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

1.1.1. Background as provided by the European Commission

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

Three applications have been introduced by the company Novozymes A/S for the authorisation of the food enzymes Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-BC), Amyloglucosidase from a genetically modified strain of *Aspergillus niger* (strain NZYM-BR) and Glucose oxidase from a genetically modified strain of *Aspergillus oryzae* (strain NZYM-KP).

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 three applications fall within the scope of the food enzyme Regulation and contain all the elements required under Chapter II of that Regulation.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-BC), Amyloglucosidase from a genetically modified strain of *Aspergillus niger*

[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.

(strain NZYM-BR) and Glucose oxidase from a genetically modified strain of *Aspergillus oryzae* (strain NZYM-KP) 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 Alpha-amylase from a genetically modified *B. licheniformis* (strain NZYM-BC).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme α-amylase from a genetically modified *B. licheniformis* (strain NZYM-BC).

Additional information was requested from the applicant during the assessment process on 8 July 2014 and on 25 November 2020 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 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	α-Amylase
Systematic name	1,4-α-D-glucan glucanohydrolase
Synonyms	Glycogenase, endoamylase, Taka-amylase
IUBMB No.	EC 3.2.1.1
CAS No.	9,000-90-2
EINECS No.	232–565-6

α-Amylases catalyse the hydrolysis of 1,4-α-glucosidic linkages in starch (amylose and amylopectin), glycogen as well as related polysaccharides and oligosaccharides, resulting in the generation of soluble dextrins and other oligosaccharides. The enzyme is intended to be used in six food manufacturing processes, namely starch processing for the production of glucose syrups and other starch hydrolysates, distilled alcohol production, brewing processes, cereal-based processes, refined and unrefined sugar production and fruit and vegetable processing for juice production.

3.1. Source of the food enzyme

The α-amylase is produced with the genetically modified bacterium *B. licheniformis* strain NZYM-BC, which is deposited in the German Collection of Microorganisms and Cell Cultures (DSMZ, Germany) with 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 the absence of acquired antimicrobial resistance genes and toxigenic activity have been verified for the specific strain used (EFSA BIOHAZ Panel, 2020). [redacted]

[4] Technical dossier/GMM dossier-Annex 4/Annex A3.
[5] Technical dossier/Additional information February 2021.

[redacted][5] The strain did not show cytotoxic activity [redacted][5] Therefore, the strain was considered to qualify for the QPS approach.

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

[redacted]

3.1.2. Characteristics of introduced sequences

[redacted][9]

3.1.3. Description of the genetic modification process

[redacted]

[6] Technical dossier/GMM dossier-Annex 4.
[7] Technical dossier/GMM dossier-Annex 4/Annexes B2-B24.
[8] Technical dossier/GMM dossier-Annex 4/Annexes B25-B35.
[9] Technical dossier/GMM dossier-Annex 4/Annexes C01-C05.

[redacted][11]

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.

[redacted][12]

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[13], with food safety procedures based on hazard analysis and critical control points, and in accordance with current good manufacturing practice.[14]

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 the enzyme protein is retained, while most of the low molecular mass material passes the filtration membrane and is discarded. Finally, the food enzyme is stabilised before formulation.[15] 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.[16]

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 α-amylase is a single polypeptide chain of 513 amino acids.[17] The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be 59 kDa. The food enzyme

[10] Technical dossier/GMM dossier-Annex 4/Annex D1.
[11] Technical dossier/GMM dossier-Annex 4/Annexes C6-C10.
[12] Technical dossier/GMM dossier-Annex 4/Annex D2.
[13] 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.
[14] Technical dossier/pp. 20/Annex 5.
[15] Technical dossier/pp. 20–24 and 73–79.
[16] Technical dossier/Annex 6.
[17] Technical dossier/pp. 58/Annex 1.

was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE analysis). A consistent protein pattern was observed across all batches with a major band migrating at ~ 49 kDa, plus some minor bands of lower mass.[18] The food enzyme was tested for protease, β-glucanase, phospholipase and glucoamylase activities; none were detected.[19]

The in-house determination of α-amylase activity is based on hydrolysis of 4,6-ethylidene(G7)-*p*-nitrophenyl(G1)-α-D-maltoheptaoside (ethylidene-G7pNP) by a coupled reaction that results in the release of *p*-nitrophenol (reaction conditions: pH 7.0, temperature 37°C, reaction time 5 min). The enzymatic activity is quantified by measuring the formation of *p*-nitrophenol spectrophotometrically at 405 nm. The activity is quantified relative to an internal enzyme standard (S) and expressed in Kilo Novo alpha-amylase Units (S)/g (KNU(S)/g).[20]

The food enzyme has a temperature optimum around 70°C (pH 4.5) and a pH optimum around pH 5 (30°C). Its thermostability was tested after a pre-incubation for 30 min at different temperatures (pH 4.5). The α-amylase activity decreased above 70°C, showing no residual activity at 100°C.[21]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation and one batch used for toxicological testing (Table 1). The mean total organic solids (TOS) of the three food enzyme batches used for commercialisation was 4.0% and the mean enzyme activity/TOS ratio 24.6 KNU(S)/mg TOS.[5]

Table 1: Compositional data of the food enzyme

Parameters	Unit	Batch			
		1	2	3	4[(a)]
α-amylase activity	KNU(S)/g batch[(b)]	1,220	632	1,130	1,760
Protein	%	3.0	2.0	3.3	7.2
Ash	%	0.6	0.4	0.4	3.1
Water	%	94.7	97.0	94.8	87.0
Total organic solids (TOS)[(c)]	%	4.7	2.6	4.8	9.9
Activity/mg TOS	KNU(S)/mg TOS	26.0	24.3	23.5	17.8

(a): Batch used for the genotoxicity studies.
(b): KNU(S): Kilo Novo alpha-amylase Units (see Section 3.3.1).
(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, 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).[5] The levels of cadmium, mercury and arsenic were below the limits of detection (LODs) of the employed methodologies.[22,23]

The food enzyme complies with the microbiological criteria (for total coliforms, *Escherichia coli* and *Salmonella*) as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[24] No antimicrobial activity was detected in any of the tested batches (FAO/WHO, 2006).[25]

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

[18] Technical dossier/page 60.
[19] Technical dossier/pp. 68.
[20] Technical dossier/pp. 63–65/Annex 3.
[21] Technical dossier/page 66–67/Annex 9.
[22] LoDs: Pb = 0.5 mg/kg; As = 0.1 mg/kg; Cd = 0.05 mg/kg; Hg = 0.03 mg/kg.
[23] Technical dossier/pp. 13–14 and 61.
[24] Technical dossier/pp. 15 and 63.
[25] Technical dossier/pp. 14 and 61.

3.3.4. Viable cells and DNA of the production strain

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

The absence of recombinant DNA in the food enzyme was demonstrated [redacted][27]

3.4. Toxicological data

The applicant provided a bacterial gene mutation assay (Ames test) and an *in vitro* micronucleus test performed with the food enzyme under assessment (batch 4, Table 1). Furthermore, a repeated dose 90-day oral toxicity study was performed with an α-amylase produced with an intermediate upstream strain in the same strain lineage as the strain NZYM-BC.

Although the production strain qualified for a QPS approach and no toxicological tests are needed, the genotoxicity tests were considered as supporting evidence. The repeated dose 90-day oral toxicity study was not considered, as it could not be ascertained that the test item was fully representative of the food enzyme under assessment.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was performed according to the Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997) and following Good Laboratory Practice (GLP).[28] Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *E. coli* WP2uvrA(pKM101) were used in the presence or absence of metabolic activation (S9-mix), applying the 'treat and plate' assay. Two separate experiments were carried out in triplicate, using six concentrations of the food enzyme (from 156 to 5,000 μg dry matter/plate, corresponding to 126, 254, 508, 1016, 2029 and 4057 μg TOS/plate). No cytotoxicity was observed at any concentration levels. Growth stimulation, as demonstrated by increases in the viable count of the exposed cultures compared to the solvent control, was observed in all the tested strains, especially in the presence of S9-mix. 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 did not induce gene mutations under the test conditions employed in this study.

3.4.1.2. *In vitro* micronucleus assay

The *in vitro* micronucleus test was carried out according to OECD Draft Guideline 487 (OECD, 2010) and following GLP.[29] A dose-range finding experiment and a main experiment were performed in duplicate cultures of human peripheral whole blood lymphocytes. In the dose range-finding experiment, cells were exposed to a range of concentrations from 18.14 to 5,000 μg food enzyme/mL, corresponding to 1.82 to 495 μg TOS/mL. No cytotoxicity was observed at any of the concentrations tested. The food enzyme was therefore tested at 3,000, 4,000 and 5,000 μg/mL, corresponding to 297, 396 and 495 μg TOS/mL. Cells were exposed for 3 h in the presence or absence of S9-mix and harvested 24 h after the beginning of the treatment (3 + 21 h treatment). Additionally, a continuous 24-h treatment without S9-mix was included with harvesting 24 h after removal of the food enzyme (24 + 24-h treatment). Cytotoxicity of 12% and 7% was observed after the continuous treatment in the absence of S9-mix at 4,000 and 5,000 μg food enzyme/mL, respectively. The frequency of binucleated cells with micronuclei (MNBN) was comparable to the negative controls at all concentrations tested.

[26] Technical dossier/GMM dossier-Annex 4/Annex E1.
[27] Technical dossier/Technical dossier/Additional information February 2021.
[28] Technical dossier/Annex 7–01.
[29] Technical dossier/Annex 7–02.

The Panel concluded that the food enzyme α-amylase did not induce an increase in the frequency of MNBNs in cultured human peripheral blood lymphocytes, under the test conditions employed in this study.

3.4.2. Allergenicity

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

The potential allergenicity of the α-amylase produced with the genetically modified *B. licheniformis* strain NZYM-BC 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 Asp o 21, an α-amylase produced by *Aspergillus oryzae* known to be an occupational respiratory allergen.[30]

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

α-Amylase from *A. oryzae* is known as an occupational respiratory allergen associated with baker's asthma (Brisman and Belin, 1991; Sander et al., 1998; Brisman, 2002; Quirce et al., 2002). However, several studies have shown that adults with occupational asthma caused by an enzyme (as described for α-amylase from *A. oryzae*) can ingest respiratory allergens without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Poulsen, 2004; Armentia et al., 2009). Considering the wide use of α-amylase as a food enzyme, only a low number of case reports have been described in the literature focused on allergic reactions upon oral exposure to α-amylase in individuals respiratorily sensitised to α-amylase (Losada et al., 1992; Quirce et al., 1992; Baur and Czuppon, 1995; Kanny and Moneret-Vautrin, 1995; Moreno-Ancillo et al., 2004).

[redacted] that may cause allergies or intolerances (listed in the Regulation (EU) No 1169/2011[31]), is used in the media fed to the microorganisms as a protein source. However, during the fermentation process, it will be 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 no potentially allergenic residues are present in the food enzyme.

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 (except for distilled alcohol production), but the likelihood of such reactions to occur was 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 six food manufacturing processes at the recommended use levels summarised in Table 2.[5]

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

Food manufacturing process[(a)]	Raw material (RM)	Recommended maximum dosage of the food enzyme (mg TOS/kg RM)[(b)]
Starch processing for the production of glucose syrups and other starch hydrolysates	Starch	3.27
Distilled alcohol production	Starch	4.90

[30] Technical Dossier/Annex 8.

[31] 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.

Food manufacturing process[(a)]	Raw material (RM)	Recommended maximum dosage of the food enzyme (mg TOS/kg RM)[(b)]
Brewing processes	Cereals (malted or unmalted)	7.84
Cereal-based processes	Flour	7.84
Refined and unrefined sugar production	Sugar beet or cane	0.016
Fruit and vegetable processing for juice production	Fruit and vegetables	0.21

(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 24.6 KNU(S)/mg TOS.

In starch processing, the α-amylase is added to starch during mixing or secondary liquefaction.[32] It degrades starch into dextrins and reduces the viscosity of the gelatinised starch. The food enzyme–TOS is removed from the final glucose syrups by treatment with activated charcoal or similar and ion-exchange resins. The same was concluded for other starch hydrolysates (EFSA CEP Panel, 2021a).

In distilled alcohol production, the α-amylase is added to starch during slurry mixing or during liquefaction.[33] It degrades starch into dextrins and reduces the viscosity of the gelatinised starch. The food enzyme–TOS is not carried over with the distilled alcohols (EFSA CEP Panel, 2021a).

In brewing processes, the α-amylase is added to cereals during mashing or to other materials (e.g. corn, rice or sorghum) during the cooking and/or liquefaction steps.[34] It degrades starch from the raw material into dextrins and fermentable sugars. The activity maintained at high temperature expands the possibility of using materials other than barley for beer making. The food enzyme–TOS remains in beer.

In cereal-based processes, the food enzyme is added to flour to reduce viscosity at elevated temperatures.[35] The food enzyme–TOS remains in the final foods produced.

For the production of refined sugar, the α-amylase is added to the raw juice during heating and/or clarifying steps to hydrolyse starch from sugar cane or sugar beet.[36] Raw sugar can be additionally treated with it during melting.[37] The enzymatic reaction increases solubility and facilities sugar crystallisation. The food enzyme–TOS is not carried over with the crystalised refined sugar, but remains in molasses as by-products (EFSA CEP Panel, 2021a).

In juice production, the food enzyme is added to fruit or vegetable mash during mashing and depectinisation to hydrolyse starch.[38] This improves the filtration rate and avoids haziness in the final products. The food enzyme–TOS remains in the juices.

Based on data provided on thermostability (see Section 3.3.1), it is expected that the α-amylase may remain active in some of the final foods.

3.5.2. Dietary exposure estimation

A dietary exposure was calculated only for food manufacturing processes where the food enzyme–TOS remains in the final foods, namely brewing processes, cereal-based processes, refined and unrefined sugar production, and fruit and vegetable processing for juice production.

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 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 one day per subject were excluded and high-level exposure/intake was calculated for only

[32] Technical dossier/p. 108.
[33] Technical dossier/p. 109.
[34] Technical dossier/p. 111.
[35] Technical dossier/p. 112.
[36] Technical dossier/p. 114.
[37] Technical dossier/p. 115.
[38] Technical dossier/p. 116.

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 A – Tables 1 and 2. For the present assessment, food consumption data were available from 41 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). The highest dietary exposure to the food enzyme–TOS was estimated to be about 0.05 mg TOS/kg bw per day in toddlers.

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)	0.001–0.014 (11)	0.004–0.030 (15)	0.004–0.020 (19)	0.003–0.011 (21)	0.001–0.010 (22)	0.001–0.006 (22)
Min–max 95th percentile (number of surveys)	0.004–0.028 (9)	0.011–0.053 (13)	0.008–0.044 (19)	0.008–0.029 (20)	0.006–0.039 (22)	0.006–0.024 (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	
Exposure to food enzyme–TOS was always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
Minor FoodEx categories found to only sporadically contain molasses were excluded from the exposure assessment	–
'Brown sugar' produced through use of cane molasses or caramelised sugar syrup was excluded, due to it being a niche product on the European market	–
The transfer of food enzyme–TOS into cane and beet molasses/syrups was assumed to be 100%	+
No distinction was made between beet molasses and cane syrups used as ingredients in foods	+/–
Use of recipe fractions in disaggregation FoodEx categories	+/–
Use of technical factors in the exposure model	+/–

Sources of uncertainties	Direction of impact
Exclusion of the following processes from the exposure assessment – Starch processing for the production of glucose syrups and other starch hydrolysates – Distilled alcohol 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 estimate the exposure to the food enzyme–TOS, in particular assumptions made on the occurrence and use levels of this specific food enzyme, is likely to have led to overestimation of the exposure.

The exclusion of two food manufacturing processes from the exposure assessment was based on > 99% TOS removal during these processes.

3.6. Margin of exposure

Since no toxicological assessment was considered necessary by the Panel, the margin of exposure was not calculated.

4. Conclusions

Based on the data provided, the outcome of the QPS assessment of the production strain and the removal of TOS during starch processing and distilled alcohol production, the Panel concluded that the food enzyme α-amylase produced with the genetically modified *B. licheniformis* strain NZYM-BC 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 (if appropriate)

Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-BC). August 2013. Submitted by Novozymes A/S.

Additional information. September 2014. Submitted by Novozymes A/S.

Additional information. February 2021. Submitted by Novozymes A/S.

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Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practice
GMM	genetically modified microorganism
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection

OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	total organic solids
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.2022.7370#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
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).