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SCIENTIFIC OPINION

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

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Abstract

The food enzyme α-amylase (1,4-α-D-glucan glucanohydrolase; EC 3.2.1.1) is produced with the genetically modified *Bacillus licheniformis* strain NZYM-AC by Novozymes A/S. The genetic modifications do not give rise to safety concerns and the production strain meets the requirements for the qualified presumption of safety (QPS) approach. The food enzyme was considered free from viable cells of the production organism and its DNA. It is intended to be used in seven food manufacturing processes: processing of cereals and other grains for the production of glucose syrups and other starch hydrolysates, cereal-based products other than baked, brewed products and distilled alcohol; processing of fruits and vegetables for the production of juices and products other than juices; production of refined and unrefined sugars. Since the residual amounts of total organic solids (TOS) are removed during two processes, dietary exposure was calculated only for the remaining five food manufacturing processes. It was estimated to be up to 0.167 mg TOS/kg body weight (bw) per day in European populations. Given the QPS status of the production strain and the lack of concerns resulting from the food enzyme manufacturing process, toxicological studies were not considered necessary. A search for similarity of the amino acid sequence of the food enzyme to known allergens was made and one match was found with a respiratory allergen. The Panel considered that the risk of allergic reactions by dietary exposure cannot be excluded (except for distilled alcohol production), but the likelihood is 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

1,4-α-D-glucan glucanohydrolase, *Bacillus licheniformis*, EC 3.2.1.1, food enzyme, genetically modified microorganism, glycogenase, α-Amylase

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 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 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.

Two applications have been introduced by the companies Novozymes A/S, for the authorisation of the food enzymes Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-AC) and Asparaginase from a genetically modified strain of *Aspergillus oryzae* (strain NZYM-SP).

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 both 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 assessment on the food enzymes Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-AC) and Asparaginase from a genetically modified strain of *Aspergillus oryzae* (strain NZYM-SP) 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 the food enzyme α-amylase from the genetically modified *B. licheniformis* strain NZYM-AC.

[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.

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-AC).

Additional information was requested from the applicant during the assessment process on 27 June 2014, 26 September 2014 and 16 March 2023 and 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 'Scientific Guidance for the submission of dossiers on Food Enzymes' (EFSA CEP Panel et al., 2021) and the guidance on the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel et al., 2023) have been followed for the evaluation of the application.

3 | ASSESSMENT

IUBMB nomenclature	α-Amylase
Systematic name	4-α-D-glucan glucanohydrolase
Synonyms	Endo-amylase, 1,4-α-D-glucan glucanohydrolase, glycogenase
IUBMB No	3.2.1.1
CAS No	9000-90-2
EINECS No	232-565-6

α-Amylases catalyse the hydrolysis of 1,4-*α*-glucosidic linkages in starch (amylose and amylopectin), glycogen and related polysaccharides and oligosaccharides, resulting in the generation of soluble dextrins and other oligosaccharides. The enzyme under assessment is intended to be used in seven food manufacturing processes: processing of cereals and other grains for the production of glucose syrups and other starch hydrolysates, cereal-based products other than baked, brewed products and distilled alcohol; processing of fruits and vegetables for the production of juices and products other than juices; production of refined and unrefined sugars.

3.1 | Source of the food enzyme

The *α*-amylase is produced with the genetically modified bacterium *B. licheniformis* strain NZYM-AC, which is deposited at the German Collection of Microorganisms and Cell Cultures (DSMZ, Germany) with the deposit number [redacted].[4] The production 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) genes and toxigenic activity are verified for the specific strain used (EFSA, 2007; EFSA BIOHAZ Panel, 2020) and the genetic modifications do not raise concerns. The whole genome sequence of the production strain was analysed for the presence of antimicrobial resistance genes with more than 80% similarity and 70% coverage available in the ResFinder and CARD AMR gene databases. No genes of concern were identified.[6] The applicant demonstrated the absence of cytotoxicity in Vero cells using a lactate dehydrogenase assay.[7]

3.1.1 | Characteristics of the parental and recipient microorganisms

The parental strain is *B. licheniformis* [redacted].[8]

[4]Technical dossier/GMM dossier-Annex 4/Annex A3.
[5]Technical dossier/Additional information June 2023/Annex A4.
[6]Technical dossier/Additional information June 2023/Annexes A5, A5.1 and A5.2.
[7]Technical dossier/Additional information June 2023/Annex A2.
[8]Technical dossier/GMM dossier-Annex 4/Annexes B2-B24.

[redacted]

[redacted][9] [redacted]

[redacted]

3.1.2 | Characteristics of introduced sequences

The sequence encoding the α-amylase [redacted]

[redacted][10] [redacted]

[redacted]

3.1.3 | Description of the genetic modification process

The purpose of the genetic modification was to enable the production strain to synthesise α-amylase [redacted]

- [redacted]
- [redacted]
- [redacted]

[redacted][11] [redacted][12]

[9]Technical dossier/GMM dossier-Annex 4/Annexes B25-B35.
[10]Technical dossier/GMM dossier-Annex 4/Annexes C01-C05.
[11]Technical dossier/GMM dossier-Annex 4/Annex D1.
[12]Technical dossier/GMM dossier-Annex 4/Annexes C6-C10.

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-AC differs from the recipient strain [redacted] in its capacity to overproduce the *α*-amylase [redacted].[13]

No issues of concern arising from the genetic modifications were identified by the Panel. As the other qualifications have been met, the production strain was considered to qualify for the QPS approach.

3.2 | Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[14], with food safety procedures based on Hazard Analysis and Critical Control Points and in accordance with current Good Manufacturing Practice.[15]

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. The filtrate containing the enzyme is 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. 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 483 amino acids. The molecular mass of the mature protein, calculated from the amino acid sequence, is around 55 kDa.[17] The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. A consistent protein pattern was observed across all batches. The gels showed a major protein band corresponding to an apparent molecular mass of about 48 kDa.[18] The food enzyme was tested for glucoamylase, protease, lipase and cellulase activities and 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, 37°C, reaction time 5 min) measured spectrophotometrically at 405 nm. The activity is quantified relative to an internal enzyme standard and expressed in Kilo Novo α-amylase Units/g (KNU(T)/g).[20]

The food enzyme has a temperature optimum around 60–70°C (pH 4.5) and a pH optimum around pH 6–7 (30°C). Thermostability was tested after incubation of the food enzyme for 30 min at different temperatures (pH 4.5). The α-amylase activity decreased above 45°C, showing no residual activity after pre-incubation at 80°C.[21]

3.3.2 | Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches intended for commercialisation (Table 1). The mean total organic solids (TOS) of the three food enzyme batches was 7.9% and the mean enzyme activity/TOS ratio was 5.1 KNU(T)/mg TOS.

[13]Technical dossier/GMM dossier-Annex 4/Annex D2.
[14]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.
[15]Technical dossier/Annex 5.
[16]Technical dossier/Annex 6, dossier p.76–78 (confidential) and Additional information June 2023/Add.info_2013-00586_Alpha-amylase from NZYM-AC - Annex.
[17]Technical dossier/Annex 1 (confidential).
[18]Technical dossier/ Dossier p. 59.
[19]Technical dossier/Dossier p.67; Additional data October 2014.
[20]Technical dossier/Dossier p. 62–64 and Annex 3.
[21]Technical dossier/Dossier p.65–66 and Annex 9.

3.3.3 | Purity

The lead content in the three commercial batches was below 5 mg/kg, which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). In addition, the levels of arsenic, cadmium and mercury were below the limits of detection (LoD) of the employed methods.[22,23]

TABLE 1 Composition of the food enzyme.

Parameters	Unit	Batches[c]		
		1	2	3
α-amylase activity	KNU(T)g[a]	299	368	539
Protein	%	6.5	7.8	10.9
Ash	%	1.6	1.8	2.4
Water	%	92.5	90.6	87.4
Total organic solids (TOS)[b]	%	5.9	7.6	10.2
Activity/TOS	KNU(T)/mg TOS	5.1	4.8	5.3

[a]KNU(T): Kilo Novo alpha-amylase Units (see Section 3.3.1).
[b]TOS calculated as 100% – % water – % ash.
[c]Technical dossier/Additional information June 2023/Annex 10.

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.[25]

The Panel considered that the information provided on the purity of the food enzyme was 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 [redacted]. No colonies were produced. A positive control was included.[26]

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

3.4 | Toxicological data

In line with the guidance available at the time of submission (EFSA CEF Panel, 2009), the applicant submitted a set of toxicological tests. Subsequently, the QPS approach was extended to include genetically modified microorganisms (EFSA CEP Panel, 2021). As the production strain meets the criteria required for QPS and no concerns arose from the production process, the toxicological tests were not considered.

3.4.1 | Allergenicity

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

The potential allergenicity of the α-amylase produced with the genetically modified *B. licheniformis* strain NZYM-AC 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

[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/Additional information June 2023/Annex 10.
[24]Technical dossier/ Additional information June 2023/Annex 10.
[25]Technical dossier/ Additional information June 2023/Annex 10.
[26]Technical dossier/GMM dossier-Annex 4/Annex E1.
[27]Technical dossier/Additional information June 2023/Annex E2 Version 2.

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 as an occupational respiratory allergen.[28]

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

α-Amylase from *A. oryzae* (Brisman & Belin, 1991; Sander et al., 1998; Quirce et al., 2002; Brisman, 2002) is known as an occupational respiratory allergen associated with baker's asthma. 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 (Armentia et al., 2009; Cullinan et al., 1997; Poulsen, 2004). Considering the wide use of α-amylase as a food enzyme, only a low number of case reports has been described in the literature focused on allergic reactions upon oral exposure to α-amylase in individuals respiratory sensitised to α-amylase (Baur & Czuppon, 1995; Kanny & Moneret-Vautrin, 1995; Losada et al., 1992; Moreno-Ancillo et al., 2004; Quirce et al., 1992).

[redacted], a product that may cause allergies or intolerances (listed in the Regulation (EU) No 1169/2011[29]), is used as raw material. In addition, [redacted], a known source of allergens, 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 from these sources are not expected to be present in the food enzyme.

The Panel considered that the risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded (except for distilled alcohol production), but the likelihood is low.

3.5 | Dietary exposure

3.5.1 | Intended use of the food enzyme

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

In starch processing, the food enzyme is added to starch during mixing or secondary liquefaction.[30] 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 et al., 2023).

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

Food manufacturing process[a]	Raw material (RM)	Recommended dosage of the food enzyme (mg TOS/kg RM)[b,c]
Processing of cereals and other grains		
Production of glucose syrups and other starch hydrolysates	Starch	4.8–15.8
Production of cereal-based products other than baked	Flour	3.5–**12.7**
Production of brewed products	Cereals (malted or unmalted)	6.2–**12.7**
Production of distilled alcohol	Starch dry matter	4.8–15.8
Processing of fruits and vegetables		
Production of juices	Fruit and vegetables	0.2–**0.5**
Production of fruit and vegetable products other than juices	Fruit and vegetables	0.2–**0.5**
Processing of plant- and fungal-derived products		
Production of refined and unrefined sugar	Sugar beet or sugar cane	0.03–**0.08**

Abbreviation: TOS, total organic solids.

[a]The description has been harmonised by EFSA according to the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel et al., 2023).

[b]Based on 5.06 KNU(T)/mg TOS.

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

[d]Additional information June 2023/ Add.info_2013-00586_Alpha-amylase from NZYM-AC - Annex.

[28]Technical dossier pg. 33–34, 95–98 and Annex 8.

[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/p.108.

In cereal-based processes, the food enzyme is added to the cereal slurry.[31] The hydrolysis by the α-amylase reduces the viscosity of the slurry, facilitating the downstream steps such as extrusion. The food enzyme–TOS remains in the final foods.

In brewing processes, the food enzyme is added to cereals during mashing or the cooking and/or liquefaction steps.[32] Together with other saccharifying enzymes, the α-amylase 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 distilled alcohol production, the food enzyme is added to starch during slurry mixing and liquefaction steps.[33] The α-amylase increases the amount of fermentable sugars for higher alcohol yields. The food enzyme–TOS is not carried over with the distilled alcohols (EFSA CEP Panel et al., 2023).

In juice production, the food enzyme is added to fruit or vegetable mash during liquefaction or mash treatment, following separation also to the raw juices during depectinisation, to hydrolyse starch.[34] The α-amylase degrades starch in the pressed juices, improving the filtration rate and preventing haze. The food enzyme–TOS remains in the juices.

For the production of other fruit and vegetable products, the enzyme is added to the crushed fruits and vegetables during the maceration step. The hydrolysis of starch reduces viscosity and the hydrolysates have higher solubility in the final products (e.g. jam, puree, paste and sauce). The food enzyme–TOS remains in the final processed food products.

In refined sugar production, the food enzyme is added to the raw juice during affination and/or clarifying steps to hydrolyse starch from sugar cane or sugar beet.[35] Raw sugar can be additionally treated during melting.[36] The hydrolytic action of the α-amylase increases solubility and facilitates sugar crystallisation. The food enzyme–TOS is not carried over with the crystalised refined sugar, but remains in molasses as a by-product (EFSA CEP Panel et al., 2023).

Based on data provided on thermostability (see Section 3.3.1) and the downstream processing step applied in the food processes, it is expected that the α-amylase is inactivated during most food manufacturing processes, but may remain active in juices, depending on the pasteurisation conditions.

3.5.2 | Dietary exposure estimation

In accordance with the guidance document (EFSA CEP Panel et al., 2021), dietary exposure was calculated only for food manufacturing processes where the food enzyme–TOS remains in the final foods: processing of cereals and other grains for the production of cereal-based products other than baked and brewed products; processing of fruits and vegetables for the production of juices and products other than juices; production of refined and unrefined sugar.

Chronic exposure to the food enzyme–TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSA CEP Panel et al., 2021). The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP Panel et al., 2023). Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period (days) and normalised for body weight (bw). This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only 1 day per subject were excluded and high-level exposure/intake was calculated for only those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011).

Table 3 provides an overview of the derived exposure estimates across all surveys. Detailed mean and 95th percentile exposure to the food enzyme–TOS per age class, country and survey, as well as contribution from each FoodEx category to the total dietary exposure are reported in Appendix A – Tables 1 and 2. For the present assessment, food consumption data were available from 48 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 26 European countries (Appendix B). The highest dietary exposure to the food enzyme–TOS was estimated to be 0.167 mg TOS/kg bw per day in infants class at the 95th percentile.

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.

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

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

	Estimated exposure (mg TOS/kg body weight per day)					
Population group	**Infants**	**Toddlers**	**Children**	**Adolescents**	**Adults**	**The elderly**
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0.003–0.045 (12)	0.012–0.044 (15)	0.009–0.031 (19)	0.006–0.023 (21)	0.004–0.015 (22)	0.003–0.011 (23)
Min–max 95th percentile (number of surveys)	0.018–0.167 (11)	0.037–0.108 (14)	0.027–0.074 (19)	0.016–0.054 (20)	0.014–0.060 (22)	0.011–0.036 (22)

Abbreviation: TOS, total organic solids.

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	+/–
Exclusion of the following processes from the exposure assessment – Starch processing for the production of glucose syrups and other starch hydrolysates – Distilled alcohol production	–

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

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

The exclusion of two food manufacturing processes from the calculation was based on > 99% TOS removal during these processes. This is not expected to have an impact on the overall estimate derived.

3.6 | Margin of exposure

Since toxicological assessment was considered unnecessary 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 absence of issues of concern arising from the production process, the Panel concluded that the food enzyme α-amylase produced with the genetically modified *B. licheniformis* strain NZYM-AC 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

Alpha-amylase from *Bacillus licheniformis* (strain NZYM-AC). June 2013. Submitted by Novozymes A/S.
Additional information. July 2014. Submitted by Novozymes A/S.
Additional information. October 2014. Submitted by Novozymes A/S.
Additional information. June 2023. Submitted by Novozymes A/S.

ABBREVIATIONS

bw	body weight
CAS	Chemical Abstracts Service
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
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
kDa	kiloDalton
LoD	limit of detection
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
QPS	qualified presumption of safety
TOS	total organic solids
WHO	World Health Organization

CONFLICT OF INTEREST

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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.

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SUPPORTING INFORMATION

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

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APPENDIX A

Dietary exposure estimates to the food enzyme–TOS in details

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

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

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

APPENDIX B

Population groups considered for the exposure assessment

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

[b] Consumption data from these pre-accession countries are not reported in Table 3 of this opinion, however, they are included in Appendix B for testing purpose.