SCIENTIFIC OPINION

efsa JOURNAL

ADOPTED: 5 July 2023

doi: 10.2903/j.efsa.2023.8160

Safety evaluation of the food enzyme α-amylase from the non-genetically modified *Bacillus licheniformis* strain T74

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Abstract

The food enzyme α-amylase (4-α-D-glucan glucanohydrolase; EC 3.2.1.1) is produced with the non-genetically modified microorganism *Bacillus licheniformis* strain T74 by Novozymes A/S. The production strain met the qualifications of the qualified presumption of safety (QPS) approach. The food enzyme is intended to be used in eight food manufacturing processes: starch processing for the production of glucose syrups and other starch hydrolysates, distilled alcohol production, refined and unrefined sugar production, brewing processes, cereal-based processes, fruit and vegetable processing for juice production, fruit and vegetable processing for products other than juices and the production of dairy analogues. Since residual amounts of total organic solids (TOS) are removed during two food processes (starch processing for the production of glucose syrups and other starch hydrolysates, distilled alcohol production), dietary exposure was calculated only for the remaining six food manufacturing processes. It was estimated to be up to 0.291 mg TOS/kg body weight per day in European populations. Since the production strain meets the requirements for the QPS approach and no issues of concern arose from the production process of the food enzyme, the Panel considered that toxicological studies were unnecessary. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and no match was found. The Panel considered that, under the intended conditions of use, 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. 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 glucanohydrolase, EC 3.2.1.1, *Bacillus licheniformis*, non-genetically modified microorganism

Requestor: European Commission

Question number: EFSA-Q-2022-00592

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Legal notice: 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: If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

Suggested citation: EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré, C., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Roos, Y., Aguilera, J., … Chesson, A., (2023). Safety evaluation of the food enzyme α-amylase from the non-genetically modified *Bacillus licheniformis* strain T74. *EFSA Journal, 21*(8), 1–14. https://doi.org/10.2903/j.efsa.2023.8160

ISSN: 1831-4732

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

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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 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, 2009) lays down the administrative, technical and toxicological data required.

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

1.1.1. Background as provided by the European Commission

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

Four applications have been submitted by the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) and by the companies 'DSM Food Specialties B. V.' and 'Amano Enzyme Inc.' for the food enzymes Bacillolysin from *Bacillus amyloliquefaciens and/or Bacillus subtilis*, Alpha-amylase from *Bacillus licheniformis*, Alpha-amylase from a genetically modified strain of *Bacillus subtilis* (strain NBA) and Alpha-amylase from *Aspergillus oryzae* (strain AE-AA) respectively.

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008[2], the Commission has verified that the four applications fall within the scope of the food enzyme Regulation and contains all the elements required under Chapter II of that Regulation.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out safety assessments on the food enzymes Bacillolysin from *Bacillus amyloliquefaciens and/or Bacillus subtilis*,

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

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

[3] Commission Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 64, 11.03.2011, pp. 15–24.

Alpha-amylase from *Bacillus licheniformis*, Alpha-amylase from a genetically modified strain of *Bacillus subtilis* (strain NBA) and Alpha-amylase from *Aspergillus oryzae* (strain AE-AA) in accordance with Article 17.3 of Regulation (EC) No 1332/2008[1] 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 *Bacillus licheniformis* submitted by AMFEP.

The application was submitted initially as a joint dossier[4] and identified as the EFSA-Q-2014-00911. During a meeting between EFSA, the European Commission and AMFEP,[5] it was agreed that joint dossiers will be split into individual data packages.

The current opinion addresses one data package originating from the former joint dossier. This data package is identified as EFSA-Q-2022-00592 and concerns the food enzyme alpha-amylase produced from the *Bacillus licheniformis* strain T74 and submitted by Novozymes A/S.

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 non-genetically modified *Bacillus licheniformis* strain T74.

2.2. Methodologies

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

The 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021a) has been followed for the evaluation of the application.

3. Assessment

IUBMB nomenclature	α-Amylase
Systematic name	4-α-D-glucan glucanohydrolase
Synonyms	1,4-α-D-glucan glucanohydrolase
IUBMB No	EC 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 malto-oligosaccharides. The enzyme under assessment is intended to be used in eight food manufacturing processes: starch processing for the production of glucose syrups and other starch hydrolysates, distilled alcohol production, refined and unrefined sugar production, brewing processes, cereal-based processes, fruit and vegetable processing for juice production, fruit and vegetable processing for products other than juices and the production of dairy analogues.

3.1. Source of the food enzyme

The α-amylase is produced with the non-genetically modified bacterium *Bacillus licheniformis* strain T74, which is deposited at the German Collection of Microorganisms and Cell Cultures (DSMZ, Germany) with the deposit number [redacted][6]

[4] Commission Implementing Regulation (EU) No 562/2012 of 27 June 2012 amending Commission Regulation (EU) No 234/2011 with regard to specific data required for risk assessment of food enzymes Text with EEA relevance OJ L 168, 28.6.2012, p. 21–23.

[5] The full detail is available at the https://www.efsa.europa.eu/en/events/event/ad-hoc-meeting-industry-association-amfep-joint-dossiers-food-enzymes

[6] Technical Dossier/Annex 8.

The production strain was identified as *B. licheniformis* by whole genome sequence (WGS) analysis,[7] [redacted].[8] The production strain was obtained by [redacted].[9]

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). The production strain T74 was shown to be not cytotoxic against Vero cells using the lactate dehydrogenase assay.[10] The WGS analysis of the production strain was interrogated for the presence of antimicrobial resistance genes and no hits were found.[11] Therefore, the production strain is considered to qualify for the QPS approach to safety assessment.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[12], with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current good manufacturing practice.[13]

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.[14] After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration.[15] The filtrate containing the enzyme is stabilised and 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.[16] 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.[17]

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 [redacted][18] The molecular mass of the mature protein, calculated from the amino acid sequence, is [redacted] kDa.[18] The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.[19] A consistent protein pattern was observed across all batches. The gel showed a protein migrating between the marker proteins of [redacted] and [redacted] kDa in all batches, consistent with the expected mass of the enzyme. The food enzyme was tested for glucoamylase, lipase and protease activities. Only protease activity was detected. No other enzyme activities were reported.[20]

The in-house determination of α-amylase activity is based on hydrolysis of 4,6-ethylidene(G7)- *p*-nitrophenyl(G1)-α-D-maltoheptaoside by a coupled reaction that results in the release of *p*-nitrophenol (reaction conditions: pH [redacted] °C, [redacted] min) measured by spectrophotometry at 405 nm. The enzyme activity is quantified relative to an internal enzyme standard and expressed in Kilo Novo α-amylase units (T)/g (KNU(T)/g).[21]

The food enzyme has a temperature optimum around 50°C (pH 4.5) and a pH optimum around pH 5.0 (30°C). The thermostability was tested after a pre-incubation of the food enzyme for 30 min at

[7] Technical Dossier/Annex 6.
[8] Technical Dossier/Annex 7.
[9] Technical Dossier/p. 40.
[10] Technical Dossier/Annex 9.
[11] Technical Dossier/Annex 10.
[12] 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.
[13] Technical Dossier/p. 44-45/Annex 11.
[14] Technical Dossier/p. 49–51.
[15] Technical Dossier/Annex 12.
[16] Technical dossier/pp. 44–52.
[17] Technical Dossier/p. 48/Annex 12.
[18] Technical Dossier/p. 33/Annex 1.
[19] Technical Dossier/p. 24.
[20] Technical dossier/Table 4 and Annexes: 3.02, 3.03, 3.04.
[21] Technical dossier/pp. 36-38/Annex 3.01.

different temperatures (pH 4.5). The enzyme activity decreased above 60°C, showing no residual activity after pre-incubation at 80°C.[22]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (Table 1).[23] The mean total organic solids (TOS) was 21.0% and the mean enzyme activity/TOS ratio was 2.7 KNU(T)/mg TOS.[24]

Table 1: Composition of the food enzyme

Parameters	Unit	Batches		
		1	2	3
α-Amylase activity	KNU(T)/g[(a)]	495	515	652
Protein	%	9.4	8.5	9.7
Ash	%	2.6	2.5	2.2
Water	%	75.8	77.3	76.6
Total organic solids (TOS)[(b)]	%	21.6	20.2	21.2
Activity/TOS	KNU(T) /mg TOS	2.3	2.6	3.1

(a): KNU(T): Kilo Novo α-amylase Units (T) (see Section 3.3.1).
(b): TOS calculated as 100% – % water – % ash.

3.3.3. Purity

The lead content of the three commercial batches was below 0.5 mg/kg,[25] 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, arsenic, cadmium and mercury contents were below the limits of quantification (LoQ) of the employed methods.[25,26]

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).[27] No antimicrobial activity was detected in any of the tested batches.[27]

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

3.4. Toxicological data

As the production strain qualifies for the QPS approach to safety assessment and no issue of concern arising from the production process of the food enzyme was identified (see Sections 3.1, 3.2 and 3.3), the Panel considered that no toxicological studies other than the assessment of allergenicity were necessary (EFSA CEP Panel, 2021a).

3.4.1. Allergenicity

The allergenicity assessment considered 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 non-genetically modified *B. licheniformis* strain T74 was assessed by comparing its amino acid sequence with those of known allergens according to the 'Scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms' (EFSA GMO Panel, 2010). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, no match was found.[28]

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

[22] Technical dossier/pp. 36-38/Annex 5.
[23] Technical dossier/p. 34/Annexes: 2.01–2.09, 3.01, 4.
[24] Technical Dossier/pg. 34.
[25] Technical dossier/p. 35/Annexes: 2.04, 4.
[26] LoQs: Pb = 0.5 mg/kg; As = 0.3 mg/kg; Cd, Hg = 0.05 mg/kg each.
[27] Technical dossier/p. 36/Annexes: 2.06–2.09, 4.
[28] Technical dossier/pp. 61-64/Annex 13.

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

[redacted] a product that may cause allergies or intolerances (listed in the Regulation (EU) No 1169/2011[29]), is used as raw material. However, during the fermentation process, this 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 no potentially allergenic residues from this source are present in the food enzyme.

The Panel considered that, under the intended conditions of use, 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 eight food processes at the recommended use levels summarised in Table 2.

Table 2: Intended uses and recommended use levels of the food enzyme as provided by the applicant[30]

Food manufacturing process[(a)]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[(b)]
Starch processing for the production of glucose syrups and other starch hydrolysates	Starch	11.4–30.3
Distilled alcohol production	Starch	11.4–30.3
Refined and unrefined sugar production	Sugar beet or sugar cane	0.06–**0.14**
Brewing processes	Cereal (malted or not)	18.9–**34.1**
Cereal-based processes	Flour	11.4–**30.3**
Fruit and vegetable processing for juice production	Fruit/vegetable	0.19–**1.5**
Fruit and vegetable processing for products other than juices	Fruit/vegetable	0.19–**1.5**
Production of dairy analogues	Cereals, nuts, seeds	11.4–**30.3**

(a): The name has been harmonised by EFSA according to the 'EC working document describing the food processes in which food enzymes are intended to be used' – not yet published at the time of adoption of this opinion.
(b): The numbers in bold were used for calculation.

In the production of glucose syrups and other starch hydrolysates, the food enzyme is added to starch during liquefaction and saccharification steps.[31] The food enzyme–TOS is removed from the final processed foods by treatment with activated charcoal or similar, and with ion-exchange resins (EFSA CEP Panel, 2021b).

In distilled alcohol production, the enzyme is added to the starch during slurry mixing and liquefaction steps.[32] The food enzyme 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, 2021b).

[29] Technical dossier/Annex 12.
[30] Technical dossier/p. 55.
[31] Technical dossier/p. 73.
[32] Technical dossier/pp. 74–75.

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.[33] The hydrolytic action of the α-amylase increases solubility and facilitates sugar crystallisation. The food enzyme–TOS is not carried over with the crystallised refined sugar, but remains in molasses as a by-product (EFSA CEP Panel, 2021b).

In brewing processes, the food enzyme is added to malt at the mashing step.[34] Together with other saccharifying enzymes, the α-amylase converts the liquefied starch to fermentable sugars. The food enzyme–TOS remains in the beer.

In the cereal-based processes, the food enzyme is added to the cereal slurry.[35] The hydrolysis by the α-amylase reduces the viscosity of the slurry, facilitating the downstream steps, such as extruding. The food enzyme–TOS remains in the final foods, such as breakfast cereals.

In juice production, the food enzyme is added to fruits and vegetables during both the mash treatment and the depectinisation step.[36] 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.[37] The hydrolysis of starch reduces viscosity and the hydrolysates have higher solubility and sweetness in the final products (e.g. jam, puree, paste and sauce). The food enzyme–TOS remains in the final processed food products.

In the production of liquid dairy analogues, the food enzyme is added to a slurry of milled plant materials to hydrolyse the gelatinised starch to reduce viscosity, allowing higher inclusion of plant materials in the plant-based beverages and the corresponding fermented semi-solid foods.[38,39] The food enzyme–TOS remains in the final processed foods.

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, 2021a), a dietary exposure was calculated only for food manufacturing processes where the food enzyme–TOS remains in the final foods: refined and unrefined sugar production, brewing processes, cereal-based processes, fruit and vegetable processing for juice production, fruit and vegetable processing for products other than juices and dairy analogues production.

Chronic exposure to the food enzyme–TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSA CEP Panel, 2021a). 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 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 43 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). The highest dietary exposure was estimated to be 0.291 mg TOS/kg bw per day in infants at the 95th percentile.

[33] Technical dossier/pp. 75–76.
[34] Technical dossier/pp. 77–78.
[35] Technical dossier/pp. 78–79.
[36] Technical dossier/pp. 79–80.
[37] Technical dossier/pp. 81–82.
[38] Technical dossier/p. 83.
[39] Technical dossier/p. 84.

Table 3: Summary of the estimated dietary exposure to the 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)	0.004–0.106 (12)	0.023–0.099 (15)	0.020–0.063 (19)	0.013–0.048 (21)	0.008–0.040 (22)	0.007–0.026 (23)
Min–max 95th (number of surveys)	0.016–0.291 (11)	0.061–0.239 (14)	0.045–0.175 (19)	0.039–0.130 (20)	0.032–0.161 (22)	0.026–0.098 (22)

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	+
For fruit and vegetable processing for products other than juices, not only puree and compote but also other foods (e.g. jam, canned fruit) were included in the calculation	+
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 two processes from the exposure assessment: – Starch processing for the production of glucose syrups and other starch hydrolysates – Distilled alcohol production	–

+: Uncertainty with potential to cause overestimation of exposure.
–: Uncertainty with potential to cause underestimation of exposure.

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

The exclusion of two food manufacturing processes from the exposure estimation was based on > 99% of TOS removal. This is not expected to impact on the overall estimate derived.

3.6. Margin of exposure

Given the QPS status of the production strain and the lack of hazards resulting from the food enzyme manufacturing process, toxicity tests were considered unnecessary by the Panel. Consequently, a margin of exposure was not calculated.

4. Conclusions

Based on the data provided, the qualification of the production strain to the QPS approach and the absence of issues of concern arising from the production process of the food enzyme, the Panel concluded that the food enzyme α-amylase produced with the non-genetically modified *Bacillus licheniformis* strain T74 does not give rise to safety concerns under the intended conditions of use.

5. Documentation as provided to EFSA

Alpha-amylase produced by *Bacillus licheniformis* strain T74. September 2022. Submitted by Novozymes A/S.

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Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organisation of the United Nations
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LoQ	limits of quantification
QPS	qualified presumption of safety
TOS	total organic solids
WGS	whole genome sequence
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 which can be found in the online version of this output under 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, Netherlands, Portugal, Slovenia
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).