SCIENTIFIC OPINION

efsa JOURNAL

ADOPTED: 5 July 2023

doi: 10.2903/j.efsa.2023.8157

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

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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 amyloliquefaciens* strain BA by HBI Enzymes Inc. The enzyme under assessment is intended to be used in six food processes: baking processes, brewing processes, distilled alcohol production, starch processing for the production of glucose syrups and other starch hydrolysates, production of dairy analogues and production of rice-based meals. Since residual amounts of total organic solids (TOS) are removed during distillation and during the production of glucose syrups and other starch hydrolysates, dietary exposure was calculated only for the remaining four food manufacturing processes. It was estimated to be up to 4.805 mg TOS/kg body weight (bw) per day in European populations. The applicant did not provide sufficient data to demonstrate that the production strain meets the qualified presumption of safety (QPS) criteria, or proof of absence of viable cells and DNA from the production organism in the food enzyme. Therefore, the Panel was not able to conclude on the safety of the microbial source. A margin of exposure could not be calculated in the absence of toxicological studies. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and two matches with respiratory allergens were found. The Panel considered that, under the intended conditions of use (other than distilled alcohol production), the risk of allergic reactions by dietary exposure cannot be excluded, but the likelihood is low. Based on the data provided, the Panel could not conclude on the safety of this food enzyme, under the intended conditions of use.

Keywords: food enzyme, α-amylase, 4-α-D-glucan glucanohydrolase, amylase, glycogenase, EC 3.2.1.1, *Bacillus amyloliquefacens*

Requestor: European Commission

Question number: EFSA-Q-2022-00514

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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., Andryszkiewicz, M., … Chesson, A., 2023. Safety evaluation of the food enzyme α-amylase from the non-genetically modified *Bacillus amyloliquefaciens* strain BA. *EFSA Journal, 21*(7), 1–14. https://doi.org/10.2903/j.efsa.2023.8157

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

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

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

1.1.1. Background as provided by the European Commission

Only food enzymes included in the 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[2] on food enzymes.

Three applications have been introduced by the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) for the authorisation of the food enzyme Alpha-amylase from *Bacillus amyloliquefaciens* and the company "Intertek Scientific & Regulatory Consultancy" for the authorisation of the food enzymes *Aspergillus* nuclease S_1 (the applicant has named the enzyme as Nuclease P1) from *Penicillium citrinum* (strain NP 11–15) and AMP deaminase from *Aspergillus oryzae* (strain DEA 262).

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 three 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 the safety assessments on the food enzymes *Aspergillus* nuclease S_1 from *Penicillium citrinum* (strain NP 11–15),

[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.

Alpha-amylase from *Bacillus amyloliquefaciens* and AMP deaminase from *Aspergillus oryzae* (strain DEA 262) in accordance with Article 17.3 of Regulation (EC) No 1332/20082 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 amyloliquefacens* submitted by the Association of Manufacturers and Formulators of Enzyme Products (AMFEP).

The application was submitted initially as a joint dossier[4] and identified as the EFSA-Q-2015-00846. During an ad hoc 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 joint dossier EFSA-Q-2015-00846. This data package, identified as EFSA-Q-2022-00514, concerns the food enzyme alpha-amylase that is produced with *B. amyloliquefacens* strain BA, submitted by HBI Enzymes Inc.

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 *B. amyloliquefacens* (strain BA).

Additional information was requested from the applicant during the assessment process on 23 February 2023 was consequently provided (see 'Documentation provided to EFSA'). However, some of the data requested 23 February 2023 were not provided. Consequently, the Panel concluded this assessment on the basis of the available data set.

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, 2021a) has 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	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. The food enzyme under assessment is intended to be used in six food processes: baking processes, brewing processes, distilled alcohol production, starch processing for the production of glucose syrups and other starch hydrolysates, production of dairy analogues and production of rice-based meals.

3.1. Source of the food enzyme

The α-amylase is produced with the non-genetically modified bacterium *Bacillus amyloliquefaciens* strain BA [redacted], which is deposited at the National Institute of Technology and Evaluation (NITE)

[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.

Biological Resource Center (Japan) with the deposit number [redacted].[6] The production strain was identified as *B. amyloliquefaciens* by whole genome sequence (WGS) analysis, with an average nucleotide identity of 99.9% with *B. amyloliquefaciens* type strain DMS7.[7] *B. amyloliquefaciens* BA was derived from *B. amyloliquefaciens* ATCC 27505 by conventional mutagenesis.[8]

The species *B. amyloliquefaciens* 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 BIOHAZ Panel, 2022). A cytotoxicity test made with culture supernatants indicated that the production strain *B. amyloliquefaciens* strain BA did not induce cell damage to CHO-K1 cells using the Lactate Dehydrogenase Assay.[9] The whole genome sequence of the production strain was interrogated for the presence of antimicrobial resistance genes using the ResFinder database with thresholds of > 90% identity and > 60% coverage. No hits were found with similarity to AMR genes.[10] The applicant did not provide sufficient information regarding the WGS data and the search for the possible presence of AMR genes in the genome of the production strain. In particular, the Panel considered that the threshold of 90% identity is set too high (above 80% as requested by the Panel) and could result in a failure to detect the presence of AMR genes in the production strain genome. The applicant was requested to provide a search in a second database, meeting this requirement. This was not provided. In addition, the Panel could not confirm the quality of the WGS data (completeness, correctness and contiguity), which is necessary to ensure an optimal search for AMR hits. Thus, the Panel was unable to conclude on the presence or absence of AMR genes. As a consequence, the qualification (absence of AMR genes) required for a QPS approach to safety assessment was not met.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[11], with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[12]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, [redacted] 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 then 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.[13] 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.[14]

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 [redacted] amino acids. The molecular mass of the protein, calculated from the amino acid sequence, is [redacted] kDa.[15] 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 single major protein band corresponding to an apparent molecular mass of about [redacted] kDa,[16] consistent with the expected mass of the enzyme. No other enzymatic activities were reported.

[6] Technical Dossier/Annex S.
[7] Technical Dossier/ADD DATA_APRIL 2023/Kostas lab _ ANI calculator (Bacillus amyloliquefaciens Strain BA).pdf.
[8] Technical Dossier/p. 4.
[9] Technical Dossier/Annex L.
[10] Technical Dossier/Annex C1 and C2.
[11] 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.
[12] Technical dossier/Dossier p., 23 and Annex D.
[13] Technical dossier/Dossier pp. 23–30 and Annex B.
[14] Technical Dossier/ADD DATA_APRIL 2023/230301_Raw materials for the production of food enzymes.pdf.
[15] Technical Dossier/ADD DATA_APRIL 2023/ CEA cover letter EFSA-Q-2022-00514.pdf/p. 3.
[16] Technical dossier/Dossier p. 33.

The in-house determination of α-amylase activity is based on hydrolysis of starch (reaction conditions: [redacted].[17] The activity is determined by measuring the release of reducing sugars using the Fehling-Lehmann-Schoorl method. The enzyme activity is expressed in Units/g. One Unit is defined as the amount of enzyme producing 1 mg of reducing sugars (as glucose) in 10 min under the conditions of the assay.[18]

The food enzyme has a temperature optimum around 70°C (pH 6) and a pH optimum around pH 6 (40°C). Thermostability was tested after a pre-incubation of the food enzyme for 15 min at different temperatures (pH 6). Enzyme activity decreased above 70°C, showing no residual activity above 90°C.[19]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme preparation were provided for three food enzyme batches for commercialisation (Table 1).[20] The mean total organic solids (TOS) of the three batches was 44.2% and the mean enzyme activity/TOS ratio was 1,179 U/mg TOS.[21]

Table 1: Composition of the food enzyme preparation

Parameters	Unit	Batches		
		1	2	3
α-Amylase activity	Units/g[(a)]	505,000	543,000	515,000
Protein	%	30.8	33.1	31.4
Ash	%	5.4	4.9	5.2
Water	%	4.2	4.0	4.6
[redacted]	%	48.0	44.1	47.0
Total organic solids (TOS)[(b)]	%	42.4	47.0	43.2
Activity/TOS	Units/mg TOS	1,191	1,155	1,192

(a): Units/g batch: α-amylase activity (see Section 3.3.1).
(b): TOS calculated as 100% – % water – % ash – % diluent.

3.3.3. Purity

The lead content in the three commercial batches was below 0.05 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).[22,23]

The food enzyme preparation 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). No antimicrobial activity was detected in any of the tested batches.[24]

No information was provided on the absence of viable cells or DNA of the production strain in the food enzyme.

3.4. Toxicological data

No toxicological tests were provided by the applicant.

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. amyloliquefaciens* strain BA was assessed by comparing its amino acid sequence with those of

[17] Technical Dossier/ADD DATA_APRIL 2023/230302_method_ASSAY_ ALPHA-AMYLASE(pH6.0).pdf.
[18] Technical dossier/Annex H.
[19] Technical dossier/Dossier pp. 36–37.
[20] Technical dossier/Dossier Table 6 pp.35 and Annexes F and I.
[21] Technical dossier/Dossier Table 6 pp.35 and Annexes F and I.
[22] LoQ: Pb = 0.05 mg/kg.
[23] Technical dossier/Dossier p.35 and Annex I.
[24] Technical dossier/Dossier Table 6 p.35 and Annexes J and I.

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, two matches were found.[25] The matching allergens were α-amylases produced by *A. oryzae* known as occupational respiratory allergens.

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

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

[redacted], products that may cause allergies or intolerances (listed in the Regulation (EU) No 1169/2011), may be used as raw materials. In addition, [redacted], known sources of allergens, may also be 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 no potentially allergenic residues from these sources 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 six food manufacturing 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[26]

Food manufacturing process[(a)]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[(b)]
Baking processes	flour	1–**100**
Brewing processes	cereals	4–**1000**
Distilled alcohol production	cereals	4–1000
Starch processing for the production of glucose syrups and other starch hydrolysates	starch	10–70
Production of dairy analogues	cereals, beans	1–**200**
Production of rice-based meals	rice	10–**70**

(a): The name 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): The numbers in bold were used for calculation.

In baking processes, the food enzyme is added to flour during the dough preparation.[27] The hydrolysis of α-amylase reduces the viscosity of the dough and increases the volume of the final products. The food enzyme–TOS remains in the baked foods.

[25] Technical dossier/pp. 40-42/Annex M.
[26] Technical dossier/p.63.
[27] Technical dossier/p.47.

In brewing processes, the food enzyme is added to malt at the mashing step.[28] Together with other saccharifying enzymes, the α-amylase converts the liquefied starch to fermentable sugars. The food enzyme–TOS remains in the beer.

In distilled alcohol production, the food enzyme is added to the cereals during mixing and to liquefied starch during saccharification and fermentation steps.[29] The food enzyme–TOS is not carried over with the distilled alcohols (EFSA CEP Panel, 2021b).

In the production of glucose syrups and other starch hydrolysates, the food enzyme is added to liquefied starch during the saccharification step, where the enzyme catalyses the degradation of starch polysaccharides into soluble dextrins.[30] 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 the production of dairy analogues, the food enzyme is added to a slurry of milled plant materials (e.g. cereals, beans).[31] The α-amylase hydrolyses the gelatinised starch at 50–80°C to reduce viscosity, allowing higher inclusion of plant materials in the plant-based beverages and the corresponding fermented semi-solid foods. The food enzyme–TOS remains in the final foods.

The food enzyme under assessment is also intended for the production of rice-based meals, where it is added to rice prior to cooking with water.[32] The α-amylase partially hydrolyses the starch in rice, thus delaying the retrogradation of starch in cooked rice. The food enzyme–TOS remains in the cooked rice.

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 enzyme is inactivated during all the food manufacturing processes shown in Table 2.

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: baking processes, brewing processes, production of dairy analogues production and production of rice-based meals.

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 4.805 mg TOS/kg bw per day for adults at the 95th percentile.

[28] Technical dossier/p.48.
[29] Technical dossier/p.49.
[30] Technical dossier/p.50.
[31] Technical dossier/p.52.
[32] Technical dossier/p.51, Additional data April 2023/Answer 7.

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)	0–0.232 (12)	0.018–0.619 (15)	0.024–0.510 (19)	0.005–0.354 (21)	0.190–1.206 (22)	0.164–0.648 (23)
Min–max 95th (number of surveys)	0–0.689 (11)	0.066–0.990 (14)	0.057–1.026 (19)	0.014–0.962 (20)	0.684–4.805 (22)	0.363–2.231 (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	+
Use of recipe fractions in disaggregation FoodEx categories	+/–
Use of technical factors in the exposure model	+/–
Exclusion of two processes from the exposure assessment: – Distilled alcohol production – Starch processing for glucose syrups and other starch hydrolysates	–

+: 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 assessment was based on > 99% of TOS removal. This is not expected to have an impact on the overall estimate derived.

3.6. Margin of exposure

In the absence of toxicological data, a margin of exposure could not be calculated.

4. Conclusions

In the absence of sufficient data to allow a QPS approach to the safety assessment (evidence of absence of AMR genes, or viable cells and DNA), or the identification of a margin of exposure (absence of toxicological studies), the Panel was unable to conclude on the safety of the food enzyme α-amylase produced with the *B. amyloliquefaciens* strain BA under the intended conditions of use.

Documentation as provided to EFSA

α-Amylase produced with *Bacillus amyloliquefacens* strain BA. August 2022. Submitted by HBI Enzymes Inc.

Additional information. April 2023. Submitted by Cambridge Environmental Assessments, part of RSK ADAS Europe (Ireland) Ltd.

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Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EC	European Commission
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LoQ	limit of quantification
QPS	qualified presumption of safety
TOS	total organic solids
WGS	whole genome sequencing
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, Republic of North Macedonia*, Serbia*, 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, Republic of North Macedonia*, Serbia*, Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Bosnia and Herzegovina*, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Montenegro*, Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Bosnia and Herzegovina*, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro*, Netherlands, Portugal, Romania, Serbia*, 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*, Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden

*: 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.

(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).