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Safety evaluation of the food enzyme α-amylase from the non-genetically modified *Bacillus amyloliquefaciens* strain LMG-S 32676

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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 LMG-S 32676 by Enmex SA de CV, a Kerry Company. The food enzyme under assessment is intended to be used in six food manufacturing processes: baking processes, brewing processes, distilled alcohol production, starch processing for the production of glucose syrups and other starch hydrolysates, refined and unrefined sugar production and yeast processing. Since residual amounts of total organic solids (TOS) are removed in distilled alcohol production and starch processing for glucose syrups production and other starch hydrolysates, the dietary exposure estimation was made only for the remaining four food processes. It was estimated to be up to 2.998 mg TOS/kg body weight per day in European populations. The production strain meets the requirements for the QPS approach. As no concerns arising from the manufacturing process were identified, the Panel considered that toxicological tests were not needed for the assessment of this food enzyme. 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 a 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, 1,4-α-D-glucan glucanohydrolase, *Bacillus amyloliquefaciens*

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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, 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 1332/20081 on food enzymes.

Five applications have been introduced by the companies "Nagase (Europa) GmbH" for the authorisation of the food enzyme Phospholipase A, from a genetically modified strain of *Streptomyces violoceoruber* (strain AS-10), "Novozymes A/S" for the authorisation of the food enzyme Glucose oxidase from *Aspergillus niger* (strain NZYM-KA), "Hayashibara Co., Ltd." for the authorisation of the food enzymes 4-d-D-4(1→4)-a-D-glucano} trehalose trehalohydrolase from *Arthrobacter ramosus* and (1→4)-a-D-glucan 1-a-D-glucosylmutase from *Arthrobacter ramosus*, and the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) for the authorisation of the food enzyme α-amylase from *Bacillus subtilis*.

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011 implementing Regulation (EC) No 1331/20082, the Commission has verified that the five 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 Phospholipase A2 from a genetically modified strain of *Streptomyces violoceoruber* (strain AS-10), Glucose oxidase from *Aspergillus niger* (strain NZYM-KA);

[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.

4-a-D-{(1→4)-a-D-glucano) trehalose trehalohydrolase from *Arthrobacter ramosus,* (1→4)-a-D-glucan 1-a-D-glucosylmutase from *Arthrobacier ramosus* and α-amylase from *Bacillus subtilis* in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of food enzyme α-amylase from the non-genetically modified (GM) *Bacillus subtilis* submitted by Association of Manufacturers and Formulators of Enzyme Products (AMFEP).

The application was submitted initially as a joint dossier[3] and identified as the EFSA-Q-year-2016-00133. During an ad hoc meeting between EFSA, the European Commission and AMFEP,[4] 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-2016-00133. This data package, identified as EFSA-Q-2022-00607, concerns the food enzyme α-amylase that is produced with *Bacillus subtilis* strain LMG S-32676 and submitted by Enmex SA de CV, a Kerry Company.

Recent data identified the production microorganism as *B. amyloliquefaciens* (Section 3.1). Therefore, this name will be used in this opinion instead of *B. subtilis*.

2. Data and Methodologies

2.1. Data

The applicant Enmex SA de CV, a Kerry Company has submitted a dossier in support of the application for authorisation of the food enzyme α-amylase from the non-genetically modified *Bacillus amyloliquefaciens* strain LMG-S 32676.

2.2. Methodologies

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

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

3. Assessment

IUBMB nomenclature	α-Amylase
Systematic name	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 oligosaccharides. 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, refined and unrefined sugar production and yeast processing.

[3] 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.

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

3.1. Source of the food enzyme

The enzyme α-amylase is produced with the non-genetically modified bacterium *Bacillus amyloliquefaciens* strain LMG S-32676 [redacted], which is deposited at the Bacteria Collection of the Ghent University Laboratory of Microbiology (Belgium) with the deposit number [redacted].[5] The production strain was identified as *B. amyloliquefaciens* species by [redacted].[6,7] *B. amyloliquefaciens* LMG S-32676 was derived from a wild-type isolate 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, 2020, 2022). A cytotoxicity test made with culture supernatants indicated that the production strain *B. amyloliquefaciens* LMG S-32676 did not induce cell damage to Vero cells using the Lactate Dehydrogenase assay.[9] WGS analysis of the production strain showed the presence of genes conferring antimicrobial resistance, but they were considered intrinsic and therefore of no safety concerns.[10] Therefore, the production strain was considered to meet the requirements 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[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] 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 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.[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 mature protein, calculated from the amino acid sequence, is [redacted] kDa.[15] The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.[16] A consistent protein pattern was observed across all batches. The gel showed a major protein band corresponding to an apparent molecular mass of about [redacted] kDa, consistent with the expected mass of the enzyme. No other enzymatic activities were reported.[17]

The in-house determination of α-amylase activity is based on the hydrolysis of soluble starch (reaction conditions: pH [redacted] min). The activity is determined by the reduction in the ability of starch to complex with iodine to produce a blue colour. It is expressed in Modified Wohlgemuth Unit

[5] Technical Dossier/Annex K.
[6] Technical Dossier/Annex J.
[7] Technical Dossier/ADD Data_May 2023/Annex A.
[8] Technical Dossier/p. 40.
[9] Technical Dossier/Annex L.
[10] Technical Dossier/Annex P.
[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/p. 47/Annex M.
[13] Technical dossier/pp. 47–53/Annex N.
[14] Technical dossier/p. 47, p. 49/Annex O; ADD Data_May 2023/Annex C.
[15] Technical dossier/p. 33/Annex T.
[16] Technical dossier/p. 35/Annex E.
[17] Technical dossier/p. 36.

(MWU)/g. One MWU is the amount of enzyme which hydrolyses 1.0 mg of soluble starch as defined by reference to a colour standard under the conditions of the assay.[18]

The food enzyme has a temperature optimum around 70°C (pH 5.4) and a pH optimum between pH 5.5 and 7.0 (40°C). Thermostability was tested after a pre-incubation of the food enzyme for 15 min at different temperatures (pH 5.4). The enzyme activity decreased above 70°C, showing no residual activity above 85°C.[19]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme preparation were provided for three batches used for commercialisation (Table 1).[20] The mean total organic solids (TOS) of the three batches was 72.0% and the mean enzyme activity/TOS ratio was 9,902 MWU/mg TOS.

Table 1: Composition of the food enzyme preparation

Parameters	Unit	Batches		
		1	2	3
α-Amylase activity	MWU/g[(a)]	7,021,000	7,019,000	7,286,000
Protein	%	32.0	34.7	27.1
Ash	%	23.0	18.0	11.0
Water	%	10.0	11.0	11.0
Total organic solids (TOS)[(b)]	%	67.0	71.0	78.0
Activity/TOS	MWU/mg TOS	10,479	9,886	9,341

(a): MWU: Modified Wohlgemuth Unit (see Section 3.3.1).
(b): TOS calculated as 100% – % water – % ash.

3.3.3. Purity

The lead content in the three commercial batches was below 1 mg/kg,[21,22] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).

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).[21] No antimicrobial activity was detected in any of the tested batches.[23]

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 in three independent batches analysed in triplicate. Ten grams of product were dissolved in 50 mL of non-selective medium; 5 mL of this solution was then filtered through a membrane (0.45 μm pore size). The membranes were placed on a plate and incubated at 34°C for 5 days. No colonies of the production strain were found.[24]

The absence of DNA in the food enzyme was demonstrated by polymerase chain reaction (PCR) analysis of three batches in triplicate. No DNA was detected with primers that would amplify a 209-bp fragment specific for the production strain, with a limit of detection of 10 ng spiked DNA/mL food enzyme.[25]

[18] Technical dossier/pp. 35–36/Annex D.
[19] Technical dossier/pp. 37–38.
[20] Technical dossier/p. 33/Annexes: A,B, C, D, G.
[21] Technical dossier/pp. 34–35/Annex G.
[22] LoD: Pb = 0.029 mg/kg.
[23] Technical dossier/pp. 34–35/Annex F.
[24] Technical Dossier/ADD Data_May 2023/Annex B1.
[25] Technical Dossier/ADD Data_May 2023/Annex B2.

3.4. Toxicological data

As the production strain qualifies for the QPS approach of safety assessment and no issue of concern arising from the production process of the food enzyme were identified (see Sections 3.1, 3.2 and 3.3), the Panel 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 *B. amyloliquefaciens* strain LMG-S 32676 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, two matches were found. The matching allergens were α-amylases produced by *A. oryzae*, known as occupational respiratory allergens.[26]

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

Several studies have shown that adults with occupational asthma due to a food enzyme (as described for α-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 α-amylase as food enzyme only a low number of case reports of allergic reactions upon oral exposure to α-amylase in individuals respiratory sensitised to α-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], that may cause allergies or intolerances (listed in the Regulation (EU) No 1169/2011), is used as a 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 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[27]

Food manufacturing process[(a)]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[(b)]
Baking processes	Flour	30–**110**
	Starch	
Brewing processes	Cereals	100–**500**
	Malt	

[26] Technical dossier/p. 66/Annex T.

Food manufacturing process[(a)]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[(b)]
Distilled alcohol production	Cereals	200–700
	Corn	
Starch processing for the production of glucose syrups and other starch hydrolysates	Cereals	200–700
	Corn	
	Potatoes	
Refined and unrefined sugar production	Sugar cane	10–**50**
	Sugar beet	
Yeast processing	Yeast cells including cell walls	400–**1,400**

(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 baking processes, the food enzyme is added to flour during the dough preparation.[28] The hydrolysis of α-amylase reduces the viscosity of the dough and increases the volume of the final product. The food enzyme–TOS remains in the baked foods.

In brewing processes, the food enzyme is added in the mashing step or to the adjunct before its transfer to the mash tun.[29] α-Amylase along with other saccharifying enzymes (e.g. β-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 liquefied starch during saccharification and fermentation steps.[30] The α-amylase increases the amounts of fermentable sugars for increased yields of alcohol. The food enzyme–TOS is not carried over with the distilled alcohols (EFSA CEP Panel, 2021a).

In starch processing for the production of glucose syrups and other starch hydrolysates, the food enzyme is added to liquefied starch at the saccharification step.[31] The food enzyme–TOS is removed in the final processed foods by treatment with activated charcoal and ion exchange resins (EFSA CEP Panel, 2021a).

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.[32] The hydrolytic action of the α-amylase 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, 2021b).

In yeast processing, the food enzyme may be added to the yeast during autolysis, to yeast extract and to yeast cell walls.[33,34] The food enzyme–TOS remains in the final processed yeast products.

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 all the food manufacturing processes listed 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, in which the food enzyme–TOS remains in the final foods: baking processes, brewing processes, refined and unrefined sugar production and yeast processing.

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 (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

[27] Technical dossier/p.62, Additional data May 2023/Responses 5 and 6.
[28] Technical dossier/p. 56.
[29] Technical dossier/p. 57.
[30] Technical dossier/p. 58.
[31] Technical dossier/p. 59.
[32] Technical dossier/p. 60.
[33] Technical dossier/p. 61.
[34] Technical dossier/Annex R.

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 was estimated to be 2.998 mg TOS/kg bw per day in adults at the 95th percentile.

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)	0.003–0.243 (12)	0.054–0.611 (15)	0.139–0.972 (19)	0.030–0.651 (21)	0.170–0.822 (22)	0.142–0.402 (23)
Min–max 95th percentile (number of surveys)	0.007–0.675 (11)	0.202–1.115 (14)	0.457–2.922 (19)	0.107–2.056 (20)	0.481–2.998 (22)	0.335–1.275 (22)

3.5.3. Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties have been considered and are summarised in Table 4.

Table 4: Qualitative evaluation of the influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction of impact
Model input data	
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/–
Use of data from food consumption surveys of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Possible national differences in categorisation and classification of food	+/–
Model assumptions and factors	
Exposure to food enzyme–TOS was always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
Minor FoodEx categories found to only sporadically contain molasses were excluded from the exposure assessment	–
Brown sugar produced through use of cane molasses or caramelised sugar syrup was excluded, due to it being a niche product on the European market	–
The transfer of food enzyme–TOS into cane and beet molasses/syrups was assumed to be 100%	+
No distinction was made between beet molasses and cane syrups used as ingredients in foods	+/–
Use of recipe fractions in disaggregation FoodEx categories	+/–
Use of technical factors in the exposure model	+/–
Exclusion of two processes from the exposure assessment: • Distilled alcohol production • Starch processing for glucose syrups production 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 estimate the exposure to food enzyme–TOS, in particular assumptions made on the occurrence and use levels of this specific food enzyme, is likely to have led to an overestimation of the exposure.

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

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

4. Conclusions

Based on the data provided, the QPS status of the production strain 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 *B. amyloliquefaciens* strain LMG S-32676 does not give rise to safety concerns under the intended conditions of use.

5. Documentation as provided to EFSA

Dossier "Alpha-amylase from the *B. amyloliquefaciens* strain LMG-S 32676". September 2022. Submitted by ENMEX SA de CV, a Kerry Company.

Additional information. May 2023. Submitted by ENMEX SA de CV, a Kerry Company.

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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
GLP	good laboratory practice
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LOD	limit of detection
MOE	margin of exposure
PCR	polymerase chain reaction
QPS	qualified presumption of safety
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TOS	total organic solids
WGS	whole genome sequencing
WHO	World Health Organization

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, Spain, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, the 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, the 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, the Netherlands, Portugal, Romania, 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, the 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, the Netherlands, Portugal, Romania, Serbia, 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).