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SCIENTIFIC OPINION

Safety evaluation of the food enzyme bacillolysin from the non-genetically modified *Bacillus amyloliquefaciens* strain AGS 430

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Abstract

The food enzyme bacillolysin (EC 3.4.24.28) is produced with the non-genetically modified *Bacillus amyloliquefaciens* strain AGS 430 by Kerry Ingredients & Flavours Ltd. The production strain qualifies for the qualified presumption of safety (QPS) approach to safety assessment. The food enzyme is intended to be used in 11 food manufacturing processes: processing of cereals and other grains for the production of baked products; cereal-based products other than baked; brewed products; starch and gluten fractions; distilled alcohol; processing of dairy products for the production of flavouring preparations and modified milk proteins; processing of meat and fish products for the production of protein hydrolysates; processing of plant- and fungal-derived products for the production of protein hydrolysates and plant-based analogues of milk and milk products; processing of yeast and yeast products. Since residual amounts of the total organic solids (TOS) are removed during two processes, dietary exposure was estimated only for the remaining nine food manufacturing processes. Exposure was estimated up to 3.482 mg TOS/kg body weight (bw) per day in European populations. As the production strain qualifies for the QPS approach and no issue of concern arose from the production process of the food enzyme, the Panel considered that no toxicological studies other than the assessment of allergenicity were necessary. 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 the risk of allergic reactions upon dietary exposure cannot be excluded (except for distilled alcohol production), but the likelihood is low. Based on the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

KEYWORDS

bacillolysin, *Bacillus amyloliquefaciens*, *Bacillus* metalloendopeptidase, EC 3.4.24.28, food enzyme

CONTENTS

1 | INTRODUCTION

Article 3 of the Regulation (EC) No 1332/2008[1] provides definition for 'food enzyme' and 'food enzyme preparation'.

'Food enzyme' means a product obtained from plants, animals or microorganisms or products thereof including a product obtained by a fermentation process using microorganisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.

'Food enzyme preparation' means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

Before January 2009, food enzymes other than those used as food additives were not regulated or were regulated as processing aids under the legislation of the Member States. On 20 January 2009, Regulation (EC) No 1332/2008 on food enzymes came into force. This Regulation applies to enzymes that are added to food to perform a technological function in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food, including enzymes used as processing aids. Regulation (EC) No 1331/2008[2] established the European Union (EU) procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

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

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

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

1.1 | Background and terms of Reference as provided by the requestor

1.1.1 | Background as provided by the European Commission

Only food enzymes included in the 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 on food enzymes.

Five applications have been introduced by the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) for the authorisation of the food enzyme Bacillolysin from *Bacillus amyloliquefaciens*, and the companies "Danisco US Inc." for the authorisation of the food enzymes Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb44), Beta-galactosidase from a genetically modified strain of *Bacillus subtilis* (DP-Ezg29) and Endo-1,4-beta-xylanase from a genetically modified strain of *Bacillus subtilis* (Dp-Ezd31), and "Intertek Scientific & Regulatory Consultancy" for the authorisation of the food enzyme Beta-Fructofuranosidase from *Aspergillus fijiensis* (strain ATCC® 20611™).

Following the requirements of Article 12.1 of Commission Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/20084, the Commission has verified that the application falls 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 Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb44), Bacillolysin from *Bacillus amyloliquefaciens*, Beta-galactosidase from a genetically modified strain of *Bacillus subtilis* (DP-Ezg29), Endo-1,4-beta-xylanase from a genetically modified strain of *Bacillus subtilis* (Dp-Ezd31) and Beta-Fructofuranosidase from *Aspergillus fijiensis* (strain ATCC® 20611™) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

[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 (EC) 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.

1.2 | Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of the food enzyme Bacillolysin from *Bacillus amyloliquefaciens*. The applicants have submitted six independent data packages corresponding to this mandate (former question number EFSA-Q-2015-00837). The current opinion addresses the food enzyme produced with strain AGS 430 submitted by Kerry Ingredients & Flavours Ltd, under a new question number (EFSA-Q-2021-00492).

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme Bacillolysin from a non-genetically modified *B. amyloliquefaciens* (strain AGS 430). The dossier was submitted in September 2021.

Additional information was requested from the applicant during the assessment process on 6 April 2022 and received on 5 October 2022 (see 'Documentation provided to EFSA'). However, some of the data requested in April 2022 were not provided. The applicant sent spontaneous data in December 2022 covering partially the data not provided in October 2022.

Following the receipt of additional data by EFSA on 5 October 2022, EFSA requested a clarification teleconference on 31 May 2023, after which the applicant provided additional data on 15 June 2023. On 2 August 2023, the applicant provided spontaneous additional data.

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 current '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, et al., 2021a).

3 | ASSESSMENT

IUBMB nomenclature	Bacillolysin
Systematic name	–
Synonyms	*Bacillus* metalloendopeptidase; *Bacillus subtilis* neutral proteinase
IUBMB No	EC 3.4.24.28
CAS No	9080-56-2
EINECS No	232-991-2

Bacillolysins catalyse the hydrolysis of peptide bonds of proteins with broad specificity, releasing peptides and amino acids. The food enzyme under assessment is intended to be used in 11 food manufacturing processes: processing of cereals and other grains for the production of baked products; cereal-based products other than baked; brewed products; starch and gluten fractions; distilled alcohol; processing of dairy products for the production of flavouring preparations and modified milk proteins; processing of meat and fish products for the production of protein hydrolysates; processing of plant- and fungal-derived products for the production of protein hydrolysates and plant-based analogues of milk and milk products; processing of yeast and yeast products.

3.1 | Source of the food enzyme

The bacillolysin is produced with the bacterium *Bacillus amyloliquefaciens* strain AGS 430, which is deposited at the Westerdijk Fungal Biodiversity Institute culture collection (the Netherlands) with the deposit number [redacted].[4] The

[4]Technical dossier/risk assessment data/ Annex VIII; Additional data October 2022.

production strain was identified as *B. amyloliquefaciens* [redacted].[5]

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 cytotoxic activity are verified for the specific strain used (EFSA, 2007; EFSA BIOHAZ Panel, 2020). The production strain was shown not to be cytotoxic [redacted].[6] [redacted]. No genes of concern were detected. 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[7], with food safety procedures based on Hazard Analysis and Critical Control Points and in accordance with current good manufacturing practice.[8]

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.[9] 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.[10]

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 bacillolysin consists of a single polypeptide chain of [redacted] amino acids.[11] The molecular mass of the mature protein following the cleavage of pre- and pro-sequences, calculated from the amino acid sequence, is around [redacted] kDa.[12] The food enzyme was analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis.[13] A consistent protein pattern was observed across all batches, with a major band migrating at around [redacted] kDa ascribed to the bacillolysin. The food enzyme was also shown to contain α-amylase activity.[14] No other enzyme activities were reported.

The in-house determination of bacillolysin activity is based on the hydrolysis of dimethylated casein (reaction conditions: [redacted]). The enzyme activity is determined by measuring the release of low molecular mass peptides by a colorimetric assay. The enzyme activity is expressed in neutral protease units (U). One unit is defined as the amount of enzyme which releases peptides from casein equivalent to 1 μg of tyrosine per minute under the conditions of the assay.[15]

The food enzyme has a temperature optimum around 50°C (pH 7.0) and a pH optimum around pH 7.0 (25°C). Thermostability was tested after a pre-incubation of the food enzyme for 15 min at different temperatures. The enzyme activity decreased above 40°C, showing no residual activity after pre-incubation at 80°C.[16]

3.3.2 | Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (Table 1).[17] The mean total organic solids (TOS) of the three food enzyme batches was 11.9% and the mean enzyme activity/TOS ratio was 832 U/mg TOS.

[5]Technical dossier/risk assessment data/ Annex IX 9.1 and 9.2.
[6]Technical dossier/risk assessment data/ Annex IX 9.4.
[7]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.
[8]Technical dossier/risk assessment data/Annex X.
[9]Technical dossier/risk assessment data/Annex XI.1; Spontaneous data December 2022/Annex XI_1.
[10]Technical dossier/risk assessment data/Annex XI.3; Additional data October 2022; Spontaneous data December 2022/Annex XI_3.
[11]Technical dossier/risk assessment data p. 19/Annex I.
[12]Technical dossier/risk assessment data p. 19, p. 21/Annex IV.
[13]Technical dossier/risk assessment data p. 21/Annex IV; Additional data October 2022.
[14]Technical dossier/risk assessment data/Annex III and Annex II.3.
[15]Technical dossier/risk assessment data/Annex II – 2.4.1 and 2.4.2.
[16]Technical dossier/risk assessment data pp. 25-27/Annex VI.
[17]Technical dossier/risk assessment data p. 20/Annex III.

TABLE 1 Composition of the food enzyme.

Parameters	Unit	Batches		
		1	2	3
Bacillolysin activity	U/mL[a]	96,241	94,200	107,450
Protein	%	3.8	4.2	5.3
Ash	%	0.6	0.7	0.7
Water	%	87.5	87.4	87.3
Total organic solids (TOS)[b]	%	11.9	11.9	12.0
Activity/TOS ratio	U/mg TOS	809	792	895

[a]U: Neutral protease unit (see Section 3.3.1).
[b]TOS calculated as 100% – % water – % ash.

3.3.3 | Purity

The lead content in the three batches was below 0.005 mg/kg[18,19], 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).[20] No antimicrobial activity was detected in any of the tested batches.[21]

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

3.4 | Toxicological data

As the production strain qualifies for the QPS approach to safety assessment and no issues 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 considers only the food enzyme and not carriers or other excipients that may be used in the final formulation.

The potential allergenicity of the bacillolysin produced with the non-genetically modified *B. amyloliquefaciens* strain AGS 430 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.[22]

No information was available on oral and respiratory sensitisation or elicitation reactions to this enzyme. In addition, no allergic reactions upon dietary exposure to any bacillolysin have been reported in the literature.[23]

[redacted] and [redacted], products that may cause allergies or intolerances (listed in the Regulation (EU) No 1169/2011[24]), are used as raw materials. In addition, [redacted], a known source of allergens, is also present in the media fed to the microorganisms. However, during the fermentation process, these products will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the microbial biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that potentially allergenic residues from these sources are not expected to be present in the food enzyme.

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

[18]Technical dossier/risk assessment data p. 20/Annex III, Annex II.11.
[19]Pb: LoQ = 0.1 mg/kg.
[20]Technical dossier/risk assessment data p. 20/Annex III, Annex II.1, Annex II.2, Annex II.9.
[21]Technical dossier/risk assessment data p. 20/Annex III, Annex V.
[22]Technical dossier/risk assessment data pp. 56–57/Annex XII.1.
[23]Technical dossier/risk assessment data/Annex XII.2.
[24]Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

3.5 | Dietary exposure

3.5.1 | Intended use of the food enzyme

The food enzyme is intended to be used in 11 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.[c]

Food manufacturing process[a]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[b]
Processing of cereals and other grains		
Production of baked products[d]	Flour	0.1–**250**
Production of cereal-based products other than baked[e]	Flour	0.1–**250**
Production of brewed products[d]	Cereals	0.1–**150**
Production of starch and gluten fractions[e]	Grain slurry or dough	Not provided
Production of distilled alcohol[d]	Cereals	0.1–150
Processing of dairy products		
Production of flavouring preparations from dairy products[f]	Dairy products (cheese, curd, etc.)	0.2–**900**
Production of modified milk proteins[g]	Whey protein, casein	1–**400**
Processing of meat and fish products		
Production of protein hydrolysates from meat & fish protein isolates[g]	Soluble animal proteins from beef, lamb, poultry, crustaceans, etc.	80–400
	Insoluble side-stream animal products (e.g. skin, bone, viscera)	1–**400**
Processing of plant- and fungal-derived products		
Production of protein hydrolysates from plants and fungi[h]	Protein isolated from plants	1–**400**
	Mycoprotein	70–300
Production of plant-based analogues of milk and milk product[i]	Cereals, legumes, nuts, oil seeds	75–**232**
Processing of yeast and yeast products[d]	Yeast, yeast extract, cell walls	16.5–**900**

[a]The name has been harmonised by EFSA according to the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023).
[b]The numbers in bold were used for calculation.
[c]Technical dossier/Risk assessment data, Additional data October 2022, Additional data June 2023, Spontaneous additional data August 2023.
[d]Technical dossier/Risk assessment data/Table 3.2.5.
[e]Additional data October 2022/Answer 7.
[f]Technical dossier/p. 49 and Additional data October 2022/Answers 8, 9, 10, 11 and 12.
[g]Additional data October 2022/Answers 8, 9, 10, 11, 12 and Annex XXV.
[h]Additional data October 2022/Answers 11, 12 and Annex XXV.
[i]Spontaneous additional data August 2023.

In baking and cereal-based processes, the food enzyme is added to flour during the preparation of the dough or batter.[25] The bacillolysin cleaves the peptide bonds in the gluten network, thus improving the rheology of the dough. The food enzyme–TOS remains in the final food products (e.g. bread, biscuits, pasta, breakfast cereals).

In brewing processes, the food enzyme is added to cereals during the mashing step.[26] The bacillolysin hydrolyses proteins in the cereals to release free amino nitrogen for the growth of the brewer's yeast during fermentation. In addition, the partial degradation of protein ensures the clarity of beer. The food enzyme–TOS remains in the beer.

For the production of starch and gluten fractions, the food enzyme is added to the grain during the handling of the dough.[27] The bacillolysin cleaves the peptide bonds in the gluten network, improving the rheology of the dough. The food enzyme–TOS is removed from the starch or gluten by repeated washing (EFSA CEP Panel, 2023).

In distilled alcohol production, the food enzyme is added during the liquefaction and fermentation steps and may also be added during slurry mixing and pre-saccharification.[28] The bacillolysin may be used to improve the yield and to enhance the access of amylolytic enzymes to the starch granules, facilitating the degradation of starch and non-starch polysaccharides into fermentable sugars. The food enzyme–TOS is not carried over to the final processed foods (EFSA CEP Panel, 2023).

[25]Additional data October/Annex XI_2.
[26]Additional data October/Annex XI_2.
[27]Additional data October/Annex XI_2.
[28]Technical dossier/Annex XI_2.

In the production of flavouring preparations from dairy products, the food enzyme is added to dairy products (e.g. curd, cheese)[29] to create distinctive sensory properties. The food enzyme–TOS remains in these enzyme-modified dairy ingredients (EMDI), which are subsequently used as an ingredient to formulate a variety of foods, such as processed cheese, cheese sauce, cheese powder, salad dressings and snacks. The food enzyme–TOS remains in those EMDIs.

To manufacture protein hydrolysates, the food enzyme is used to treat proteins isolated from milk (e.g. whey proteins, caseins), plants (e.g. soy, wheat, maize), fungal or animal sources (e.g. meat, fish, collagen, gelatin).[30] The hydrolysis by bacillolysin increases the yield of the protein hydrolysates. The 'animal protein hydrolysates' are subjected to a second processing step of thermal treatment in the presence of added carbohydrates and amino acids to generate meat-based flavourings.[31] The food enzyme–TOS remains in the final protein hydrolysates, which are subsequently used as an ingredients in a variety of foods, including infant formula, follow-on formula and foods for special medical purposes.[32]

In the production of plant-based analogues of milk and milk products, the food enzyme is added to the slurry of plant materials (e.g. oat flour) during the saccharification step.[33] The hydrolysis by bacillolysin increases the yield and enhances the flavour. The food enzyme–TOS remains in these plant-based analogues of milk and milk products.

In yeast processing, the food enzyme is added to yeast cultures, yeast extracts or yeast cell walls during different stages of the process.[34] The bacillolysin is used to hydrolyse the insoluble proteins, optimising the extraction process and improving the functional properties of the yeast products. The food enzyme–TOS remains in these yeast products.

Based on data provided on thermostability (see Section 3.3.1) and the downstream processing steps applied in the food manufacturing processes, it is expected that this bacillolysin will be inactivated or removed in 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, 2021b), a dietary exposure was calculated only for the nine food manufacturing processes where the food enzyme–TOS remains in the final foods: processing of cereals and other grains for the production of baked products; cereal-based products other than baked; brewed products; processing of dairy products for the production of flavouring preparation and modified milk proteins; processing of meat and fish products for the production of protein hydrolysates; processing of plant- and fungal-derived products for the production of protein hydrolysates and plant-based analogues of milk and milk products; processing of yeast and yeast products.

Chronic exposure to the food enzyme–TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSA CEP Panel, 2021a, 2021b). The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP Panel, 2023). 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 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 3.482 mg TOS/kg bw per day in infants at the 95th percentile.

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

	Estimated exposure (mg TOS/kg body weight per day)					
Population group	**Infants**	**Toddlers**	**Children**	**Adolescents**	**Adults**	**The elderly**
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥65 years
Min–max mean (number of surveys)	0.424–1.464 (12)	0.847–1.788 (15)	0.363–1.470 (19)	0.106–1.031 (21)	0.332–0.677 (22)	0.291–0.608 (23)
Min–max 95th percentile (number of surveys)	1.150–3.482 (11)	1.617–2.834 (14)	0.790–2.688 (19)	0.256–1.810 (20)	0.662–1.496 (22)	0.589–1.138 (22)

[29]Technical dossier/Annex 11/11_2.

[30]Technical dossier/Annex 11/11_2, Additional data October 2022/Answers 8, 9, 10, 11, 12 and Annex XXV.

[31]Additional data June 2023.

[32]Additional data October 2022/Answer 12 and Annex XXV.

[33]Spontaneous additional data August 2023.

[34]Technical dossier/Annex 11/11_2.

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 food manufacturing processes from the exposure estimation: – production of starch and gluten fractions – production of distilled alcohol	–

Abbreviations: +, 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 during these processes and 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 | CONCLUSION

Based on the data provided, the QPS status of the production strain and the absence of issues of concern arising from the production process, the Panel concluded that the food enzyme bacillolysin produced with the non-genetically modified *Bacillus amyloliquefaciens* strain AGS 430 does not give rise to safety concerns under the intended conditions of use.

5 | DOCUMENTATION AS PROVIDED TO EFSA

Bacillolysin from *Bacillus amyloliquefaciens*. Month 2021. Submitted by Kerry Ingredients & Flavours Ltd.
Additional information. October 2022. Submitted by Kerry Ingredients & Flavours Ltd.
Spontaneous information. December 2022. Submitted by Kerry Ingredients & Flavours Ltd.
Additional information. June 2023. Submitted by Kerry Ingredients & Flavours Ltd.
Spontaneous information. August 2023. Submitted by Kerry Ingredients & Flavours Ltd.

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	limit of quantification
TOS	total organic solids
WHO	World Health Organization

CONFLICT OF INTEREST

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NOTE

The full opinion will be published in accordance with Article 12 of Regulation (EC) No 1331/2008 once the decision on confidentiality will be received from the European Commission.

REFERENCES

EFSA (European Food Safety Authority). (2006). Opinion of the Scientific Committee related to uncertainties in dietary exposure assessment. *EFSA Journal*, *5*(1), 438. https://doi.org/10.2903/j.efsa.2007.438

EFSA (European Food Safety Authority). (2007). Introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA - Opinion of the Scientific Committee. *EFSA Journal*, *5*(12), 587. https://doi.org/10.2903/j.efsa.2007.587

EFSA (European Food Safety Authority). (2009a). Guidance of EFSA prepared by the scientific panel of food contact material, enzymes, Flavourings and processing aids on the submission of a dossier on food enzymes. *EFSA Journal*, *7*(8), 1305. https://doi.org/10.2903/j.efsa.2009.1305

EFSA (European Food Safety Authority). (2009b). Guidance of the scientific committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: general principles. *EFSA Journal 2009*, *7*(5), 1051. https://doi.org/10.2903/j.efsa.2009.1051

EFSA (European Food Safety Authority). (2011). Use of the EFSA comprehensive European food consumption database in exposure assessment. *EFSA Journal*, *9*(3), 2097. https://doi.org/10.2903/j.efsa.2011.2097

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards). (2020). Scientific opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA (2017–2019). *EFSA Journal*, *18*(2), 5966. https://doi.org/10.2903/j.efsa.2020.5966

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids). (2019). Statement on the characterisation of microorganisms used for the production of food enzymes. *EFSA Journal*, *17*(6), 5741. https://doi.org/10.2903/j.efsa.2019.5741

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., Glandorf, B., Herman, L., … Chesson, A. (2021a). Scientific guidance for the submission of dossiers on food enzymes. *EFSA Journal*, *19*(10), 6851. https://doi.org/10.2903/j.efsa.2021.6851

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., Liu, Y., & Chesson, A. (2021b). Statement on the process-specific technical data used in exposure assessment of food enzymes. *EFSA Journal*, *19*(12), 7010. https://doi.org/10.2903/j.efsa.2021.7010

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes, 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., Apergi, K., … Chesson, A. (2023). Food manufacturing processes and technical data used in the exposure assessment of food enzymes. *EFSA Journal*, *21*(7), 8094. https://doi.org/10.2903/j.efsa.2023.8094

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2010). Scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. *EFSA Journal*, *8*(7), 1700. https://doi.org/10.2903/j.efsa.2010.1700

FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization). (2006). General specifications and considerations for enzyme preparations used in food processing in compendium of food additive specifications. 67th meeting. *FAO JECFA Monographs*, *3*, 63–67. https://www.fao.org/3/a-a0675e.pdf

SUPPORTING INFORMATION

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

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APPENDIX A

Dietary exposure estimates to the food enzyme–TOS in details

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

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

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

APPENDIX B

Population groups considered for the exposure assessment

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From 12 weeks on up to and including 11 months of age	Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia, Spain
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Netherlands, Portugal, Republic of North Macedonia[b], Serbia[b], Slovenia, Spain
Children	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Republic of North Macedonia[b], Serbia[b], Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Bosnia and Herzegovina[b], Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Montenegro[b], Netherlands, Portugal, Romania, Serbia[b], Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Bosnia and Herzegovina[b], Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro[b], Netherlands, Portugal, Romania, Serbia[b], Slovenia, Spain, Sweden
The elderly[a]	From 65 years of age and older	Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro[b], Netherlands, Portugal, Romania, Serbia[b], Slovenia, Spain, Sweden

[a]The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011).

[b]Consumption data from these pre-accession countries are included for testing purpose.