Adopted: 25 October 2023

DOI: 10.2903/j.efsa.2023.8391

SCIENTIFIC OPINION

efsa JOURNAL

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

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Abstract

The food enzyme bacillolysin (EC 3.4.24.28) is produced with the non-genetically modified *Bacillus amyloliquefaciens* strain GNP by DSM Food Specialties B.V. The production strain qualifies for the qualified presumption of safety (QPS) approach to safety assessment. The food enzyme is intended to be used in nine food manufacturing processes: processing of cereals and other grains for the production of baked products, cereal-based products other than baked, brewed products and distilled alcohol; 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. Since the food enzyme–total organic solids (TOS) is not carried into distilled alcohols, dietary exposure was estimated only to the remaining eight food processes. Exposure was estimated to be up to 17.934 mg TOS/kg body weight per day in European populations. As the production strain qualifies for the QPS approach to safety assessment and no issue of concern arose from the production process, no toxicological studies other than the assessment of allergenicity were required. 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, 2009) lays down the administrative, technical and toxicological data required.

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

1.1.1 | Background as provided by the European Commission

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

Five applications have been introduced by the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) for the authorization of the food enzyme Bacillolysin from *Bacillus amyloliquefaciens*, and the companies "Danisco US Inc." for the authorization 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 authorization of the food enzyme Beta-fructofuranosidase from *Aspergillus fijiensis* (strain ATCC® 20611™).

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

1.1.2 | Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments of the food enzymes Bacillolysin from *Bacillus amyloliquefaciens*, Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb44), 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 (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.

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 Bacillolysin from *Bacillus amyloliquefaciens* submitted by Association of Manufacturers and Formulators of Enzyme Products (AMFEP).

The application was submitted initially as a joint dossier[4] and identified as the EFSA-2015-00837. During the risk assessment phase, it was found that the technical dossier is too generic to be evaluated. A solution was found on 16 March 2020 via an ad-hoc meeting between EFSA, the European Commission and representatives from AMFEP.[5] It was agreed that joint dossiers will be split into six individual data packages.

The current opinion addresses one data package originating from the joint dossier EFSA-2015-00837. This data package, identified as EFSA-Q-2021-00645, concerns the food enzyme Bacillolysin that is produced with a strain of *B. amyloliquefaciens* and submitted by DSM Food Specialties B.V.

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 GNP). The dossier was submitted on 29 October 2021.

Additional information was requested from the applicant during the assessment process on 26 January 2022 and on 15 December 2022, and received on 26 September 2022 and on 26 June 2023, respectively (see 'Documentation provided to EFSA').

2.2 | Methodologies

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

The 'Scientific Guidance for the submission of dossiers on Food Enzymes' (EFSA CEP Panel et al., 2021) and the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel et al., 2023) have been followed for the evaluation of the application.

3 | ASSESSMENT

IUBMB nomenclature	Bacillolysin
Systematic name	–
Synonyms	*Bacillus* metalloendopeptidase; *Bacillus subtilis* neutral proteinase
IUBMB No	EC3.4.24.28
CAS No	9080-56-2
EINECS No	232-991-2

Bacillolysins catalyse the hydrolysis of the peptide bonds of proteins with broad specificity, releasing peptides and amino acids. The food enzyme under assessment is intended to be used in nine food manufacturing processes: processing of cereals and other grains for the production of baked products, cereal-based products other than baked, brewed products and distilled alcohol; 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.

[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, pp. 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

3.1 | Source of the food enzyme

The bacillolysin is produced with the non-genetically modified bacterium *B. amyloliquefaciens* strain GNP (DS 28443, DS 86385), which is deposited at the Westerdijk Fungal Biodiversity Institute culture collection (the Netherlands) with the deposit number [redacted].[6] The production strain was identified as *B. amyloliquefaciens* [redacted].[7]

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].[8] [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,[9] with food safety procedures based on Hazard Analysis and Critical Control Points and in accordance with current Good Manufacturing Practice.[10]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, batch 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.[11] 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.[12]

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 is a single polypeptide chain of [redacted] amino acids.[13] The molecular mass of the mature protein following the cleavage of pre- and pro-sequences, calculated from the amino acid sequence, is 32.7 kDa.[14] The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. A consistent protein pattern was observed across all batches. The gel showed a major protein band corresponding to an apparent molecular mass of about 36.5 kDa, consistent with the expected mass of the enzyme.[15] No other enzymatic activities were reported.[16]

The in-house determination of bacillolysin activity is based on the hydrolysis of casein (reaction conditions: pH 7.0, 37°C), measuring the release of peptides spectrophotometrically at 275 nm after precipitation of the unreacted casein. The bacillolysin activity is expressed in protease casein total (PCT) units. One PCT is defined as the amount of enzyme which produces an amount of peptides with an optical density equal to that of a 1.5 µg/mL tyrosine solution under the conditions of the assay.[17]

The food enzyme has a temperature optimum between 50°C and 55°C (pH 7.0) and a pH optimum around pH 7.5 (37°C). Thermostability was tested after a pre-incubation of the food enzyme at different temperatures. The bacillolysin activity decreased above 60°C, showing no residual activity above 70°C after 2 min of pre-incubation.[18]

[6]Technical dossier/p. 38/Annex I-6.
[7]Technical dossier/p. 38/Annex I-5.
[8]Technical dossier/p. 41/Annex I-7; Additional data September 2022/Part II.
[9]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.
[10]Technical dossier/p. 43/Annex I-9.
[11]Technical dossier/pp. 43-51/Annex I-10.
[12]Technical dossier/Annex I-11.
[13]Technical dossier/p. 33/Annex I-5.
[14]Technical dossier/p. 33.
[15]Technical dossier/pp. 30–31, p. 33.
[16]Technical dossier/pp. 34–35.
[17]Technical dossier/p. 34/Annex I-2.
[18]Technical dossier/pp. 35–36.

3.3.2 | Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (Table 1).[19] The mean total organic solids (TOS) of the three food enzyme batches was 19.4% and the mean enzyme activity/TOS ratio was 1753 U/mg TOS.

TABLE 1 Composition of the food enzyme.

		Batches		
Parameters	**Unit**	**1**	**2**	**3**
Bacillolysin activity	PCT/g[a]	430,000	182,500	435,500
Protein	%	9.0	5.1	8.8
Ash	%	0.7	0.8	0.2
Water	%	76.8	83.3	80.0
Total organic solids (TOS)[b]	%	22.5	15.9	19.8
Activity/TOS ratio	PCT/mg TOS	1911	1148	2200

[a]PCT: protease casein total units (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 5 mg/kg, which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[20,21]

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).[22] 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 was demonstrated [redacted]. A positive control was included.[24]

The absence of DNA in the food enzyme was demonstrated [redacted].[25]

3.4 | Toxicological data

As the production strain qualifies for the QPS approach to safety assessment and no issue of concern arising from the production process of the food enzyme 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 et al., 2021).

3.4.1 | Allergenicity

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

The potential allergenicity of the bacillolysin produced with the non-genetically modified *B. amyloliquefaciens* strain GNP was assessed by comparing its amino acid sequence with those of known allergens according to the 'Scientific

[19]Technical dossier/p. 30/Annex I-1, Annex I-2, Annex I-3.
[20]Technical dossier/p. 33/Annex I-3, Annex I-4; Additional data September 2022/Part I.
[21]LoDs: Pb = 0.7, 2.0 and 0.001 mg/kg in each batch.
[22]Technical dossier/p. 33/Annex I-3, Annex I-4.
[23]Technical dossier/p. 33/Annex I-3, Annex I-4.
[24]Additional data September 2022/Annex 1.
[25]Additional data September 2022/Annex 2.

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.[26]

No information was available on oral and respiratory sensitisation or elicitation reactions of this bacillolysin. In addition, no allergic reactions upon dietary exposure to any bacillolysin have been reported in the literature.[27]

[redacted] concentrate and [redacted], known sources of allergens, are present in the media fed to the microorganism. 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 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.

3.5 | Dietary exposure

3.5.1 | Intended use of the food enzyme

The food enzyme is intended to be used in nine 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	Flour	0.1–**250**
Production of cereal-based products other than baked	Flour	0.1–**250**
Production of brewed products	Cereals	0.1–**150**
Production of distilled alcohol	Cereals	0.1–150
Processing of dairy products		
Production of flavouring preparation from dairy products	Dairy products (cheese, curd, etc)	0.2–**900**
Production of modified milk proteins	Whey protein, casein	0.5–**6145**
Processing of meat and fish products		
Production of protein hydrolysates from meat and fish proteins	Protein isolates from animals	0.5–**2643**
Processing of plant- and fungal-derived products		
Production of protein hydrolysates from plants and fungi	Protein isolates from plants	0.5–**2643**
Production of plant-based analogues of milk and milk products	Cereals, legumes, nuts, oil seeds	9.1–**183.3**

Abbreviation: TOS, total organic solids.

[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 et al., 2023).

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

[c]Technical dossier/p. 61; Additional data September 2022/Answers 5, 6, 7, 8, 9, 10.

In baking and cereal-based processes, the food enzyme is added to flour during the preparation of the dough or batter.[28] The bacillolysin cleaves the peptide bonds in the gluten network, thus, improving rheology of the dough. The food enzyme–TOS remains in the final food products (e.g. bread, cakes, biscuits, breakfast cereals).

In brewing, the food enzyme is added to cereals during the mashing step.[29] It hydrolyses proteins in the cereals to release free amino nitrogen for the optimal growth of the brewer's yeast during fermentation. In addition, the partial degradation of protein ensures the clarity of beer.[30] The food enzyme–TOS remains in the beer.

In distilled alcohol production, the food enzyme is applied during the liquefaction and fermentation steps and may also be added during slurry mixing and pre-saccharification.[31] The bacillolysin hydrolyses proteins in the cereals to release free

[26]Technical dossier/pp. 67–69/Annex I-17.

[27]Additional data September 2022/Part I/Annex 3.

[28]Additional data September 2022/Answer 5.

[29]Technical dossier/p. 54.

[30]Technical dossier/pp. 73–74.

[31]Technical dossier/p. 56.

amino nitrogen for the optimal growth of the brewer's yeast during fermentation. The food enzyme–TOS is not carried over to the final processed foods (EFSA CEP Panel et al., 2023).

In the production of flavouring preparations from dairy products, the food enzyme is added to dairy products (e.g. curd, cheese) to create distinctive sensory properties.[32] The food enzyme–TOS remains in these enzyme modified dairy ingredients (EMDI), which are subsequently used to formulate a variety of foods, such as processed cheese, cheese sauce, cheese powder, salad dressing and snacks. The food enzyme–TOS remains in those EMDIs.

In the production of protein hydrolysates from different sources, the food enzyme is used to treat proteins isolated from milk (e.g. whey proteins, caseins), plant (e.g. soy, wheat, maize), fungal or animal sources (e.g. meat fish, collagen, gelatin).[33] Bacillolysin is used to achieve hydrolysis and enhance flavour of the resulting protein hydrolysates[34], which are subsequently used as ingredients in a variety of foods, including infant formula, follow-on formula and foods for special medical purposes.[35] The food enzyme–TOS remains in these protein hydrolysates.

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.[36] It can also be added to plant-based beverages prior to fermentation to produce fermented plant-based analogues. The bacillolysin is used to increase the yield and to enhance flavours. The food enzyme–TOS remains in these plant-based analogues.

Based on data provided on thermostability (see Section 3.3.1) and the downstream processing step 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 et al., 2021), a dietary exposure was calculated only for food manufacturing processes where the food enzyme–TOS remains in the final foods: processing of cereals and other grains for the production of baked products, cereal-based products other than baked and brewed products; processing of dairy products for the production of flavouring preparation from dairy products 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.

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

Table 3 provides an overview of the derived exposure estimates across all surveys. Detailed mean and 95th percentile exposure to the food enzyme–TOS per age class, country and survey, as well as contribution from each FoodEx category to the total dietary exposure are reported in Appendix A – Tables 1 and 2. For the present assessment, food consumption data were available from 48 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 26 European countries (Appendix B). The highest dietary exposure was estimated to be 17.934 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.

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)	0.719–7.545 (12)	1.227–5.402 (15)	0.801–2.056 (19)	0.208–1.360 (21)	0.399–0.839 (22)	0.317–0.828 (23)
Min–max 95th percentile (number of surveys)	2.199–17.934 (11)	2.711–13.408 (14)	1.538–3.574 (19)	0.462–2.653 (20)	0.763–1.863 (22)	0.632–1.623 (22)

Abbreviation: TOS: total organic solids.

[32]Technical dossier/p. 55.
[33]Technical dossier/pp. 79–80.
[34]Technical dossier/p. 79; Additional data September 2022/Answer 8.
[35]Technical dossier/p. 59; Additional data September 2022/Answer 6.
[36]Technical dossier/p. 58.

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 one process from the exposure assessment – Production of distilled alcohol	–

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

The conservative approach applied to 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 one food manufacturing process 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 *B. amyloliquefaciens* strain GNP does not give rise to safety concerns under the intended conditions of use.

5 | DOCUMENTATION AS PROVIDED TO EFSA

Bacillolysin from *Bacillus amyloliquefaciens*. October 2021. Submitted by DSM Food Specialties B.V.

Additional information. September 2022. Submitted by DSM Food Specialties B.V.

Additional information. June 2023. Submitted by DSM Food Specialties B.V.

ABBREVIATIONS

bw	body weight
CAS	Chemical Abstracts Service
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LoD	limit of detection
TOS	total organic solids
WHO	World Health Organization

CONFLICT 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.

REQUESTOR

European Commission

QUESTION NUMBER

EFSA-Q-2021-00645

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NOTE

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

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SUPPORTING INFORMATION

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

How to cite this article: 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., Herman, L., Aguilera, J., … Chesson, A. (2023). Safety evaluation of the food enzyme bacillolysin from the non-genetically modified *Bacillus amyloliquefaciens* strain GNP. *EFSA Journal*, *21*(11), e8391. https://doi.org/10.2903/j.efsa.2023.8391

APPENDIX A

Dietary exposure estimates to the food enzyme–TOS in details

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

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

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

APPENDIX B

Population groups considered for the exposure assessment

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

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

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