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SCIENTIFIC OPINION

Safety evaluation of a food enzyme containing bacillolysin and subtilisin activities from the non-genetically modified *Bacillus amyloliquefaciens* strain AR-383

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Abstract

The food enzyme with two declared activities, bacillolysin (EC 3.4.24.28) and subtilisin (EC 3.4.21.62), is produced with the non-genetically modified *Bacillus amyloliquefaciens* strain AR-383 by AB Enzymes GmbH. The food enzyme is intended to be used in nine food manufacturing processes. Since residual amounts of total organic solids (TOS) are removed in the production of distilled alcohol, dietary exposure was calculated only for the remaining eight food manufacturing processes. Exposure was estimated to be up to 1.958 mg TOS/kg body weight per day in European populations. As the production strain qualifies for the qualified presumption of safety approach to safety assessment and no issues of concern arising from the production process of the food enzyme were identified, 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 30 matches were found, including one food allergen (melon). The Panel considered that, under the intended conditions of use, the risk of allergic reactions by dietary exposure to this food enzyme cannot be excluded, but for individuals sensitised to melon, this would not exceed the risk of consuming melon. 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*, EC 3.4.21.62, EC 3.4.24.28, food enzyme, subtilisin

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; and
- 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.

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 1332/20082 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 Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/20083, 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 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 1332/20082 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 the Association of Manufacturers and Formulators of Enzyme Products (AMFEP).

The application was submitted initially as a joint dossier[4] and identified as the EFSA-Q-2015-00837. During a meeting between EFSA, the European Commission and AMFEP[5] it was agreed that joint dossiers will be split into individual data packages.

The current opinion addresses one data package originating from the joint dossier EFSA-Q-2015-00837. This data package, identified as EFSA-Q-2022-00610, concerns the food enzyme bacillolysin that is produced with a strain of *Bacillus amyloliquefaciens* and submitted by AB Enzymes GmbH.

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme containing bacillolysin and subtilisin activities from the non-genetically modified *Bacillus amyloliquefaciens* strain AR-383.

Additional information was requested from the applicant during the assessment process on 26 April 2023 and received on 3 July 2023 (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, 2009a) 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, 2009b) 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. Additional information was requested in accordance with the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021) and the guidance on the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023).

3 | ASSESSMENT

The food enzyme under application contains two declared activities:

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

Abbreviations: CAS, Chemical Abstracts Service; EINECS, European Inventory of Existing Commercial Chemical Substances; IUBMB, International Union of Biochemistry and Molecular Biology.

Bacillolysins catalyse the hydrolysis of peptide bonds of proteins with broad specificity, and with preference for Leu > Phe, releasing peptides and amino acids.

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

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

IUBMB nomenclature	Subtilisin
Systematic name	–
Synonyms	Alcalase; bacillopeptidase; colistinase; *Bacillus subtilis* alkaline proteinase; protease S
IUBMB no	EC 3.4.21.62
CAS no	9014-01-1
EINECS no	232–752-2

Abbreviations: CAS, Chemical Abstracts Service; EINECS, European Inventory of Existing Commercial Chemical Substances; IUBMB, International Union of Biochemistry and Molecular Biology.

Subtilisins catalyse the hydrolysis of peptide bonds of proteins with a broad specificity, releasing peptides and amino acids.

The enzyme under assessment is intended to be used in nine food manufacturing processes as described in the EFSA guidance (EFSA CEP Panel, 2023): processing of cereals and other grains for the production of (1) baked products, (2) cereal-based products other than baked, (3) brewed products and (4) distilled alcohol; processing of dairy products for the production of (5) flavouring preparations and (6) modified milk proteins; (7) processing of plant- and fungal-derived products for the production of protein hydrolysates; (8) processing of meat and fish products for the production of protein hydrolysates and (9) processing of yeast and yeast products.

3.1 | Source of the food enzyme

The food enzyme is produced with the non-genetically modified bacterium *Bacillus amyloliquefaciens* strain AR-383 ([redacted]), 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* by whole genome sequence (WGS) analysis, showing an average nucleotide identity [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 toxigenic activity are verified for the specific strain used (EFSA, 2007; EFSA BIOHAZ Panel, 2022). The production strain *B. amyloliquefaciens* AR-383 was found not to be cytotoxic to Vero cells using a lactate dehydrogenase assay.[8] The WGS of the production strain was interrogated for the presence of AMR genes using two regularly updated databases with thresholds of > 80% identity and > 70% coverage. No genes of concern were identified.[9]

Therefore, the production strain was considered to qualify 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,[10] with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[11]

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.[12] 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.[13]

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.

[6]Technical dossier/Annex 1.

[7]Technical dossier/Additional data July 2023/Annex 1.

[8]Technical dossier/Annex 3.

[9]Technical dossier/Annex 2/Appendixes 1,2 and additional data July 2023/Annex 1.

[10]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.

[11]Technical dossier/p. 15.

[12]Technical dossier/pp. 15–23/Annex 5.

[13]Technical dossier/p. 15, p. 18/Annex 6.

3.3 | Characteristics of the food enzyme

3.3.1 | Properties of the food enzyme

The bacillolysin and the subtilisin are single polypeptide chains of [redacted] amino acids, respectively.[14] The molecular masses of the mature proteins, calculated from their amino acid sequences, are [redacted], respectively.[15] The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. A consistent protein pattern was observed across all batches. The gel showed the target proteins corresponding to apparent molecular masses of about [redacted] and of about [redacted].[16] No other enzymatic activities were reported.[17]

The in-house determination of enzyme activity is based on the hydrolysis of [redacted] (reaction conditions: [redacted] for bacillolysin or [redacted] for subtilisin, [redacted]) and spectrophotometrically measuring the release of [redacted] hydrolysis products at 280 nm. The enzyme activity is expressed in Units of protease (UHb)/g. One UHb is defined as the amount of enzyme-releasing [redacted]-soluble [redacted] products equivalent to 1 µmol [redacted] per minute under the conditions of the assay.[18]

The food enzyme has an optimum temperature around [redacted] ([redacted] for bacillolysin and [redacted] for subtilisin). The optimum pH is between [redacted] for bacillolysin ([redacted]), and between [redacted] for subtilisin ([redacted]). Thermostability was tested after a pre-incubation of the food enzyme for [redacted] at 80°C for different times ([redacted] for bacillolysin and [redacted] for subtilisin). No enzyme activity was detected after 2 min at 80°C.[19]

3.3.2 | Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches (Table 1).[20] The mean total organic solids (TOS) was 14.85%, and the mean enzyme activity/TOS ratio was 12.590 UHb/mg TOS (bacillolysin) and 5.171 UHb/mg TOS (subtilisin).

TABLE 1 Composition of the food enzyme.

		Batches		
Parameters	**Unit**	**1**	**2**	**3**
Bacillolysin activity	UHb/mg[a]	1.924	1.801	1.817
Subtilisin activity	UHb/mg	0.754	0.749	0.765
Protein	%	7.53	6.91	6.59
Ash	%	1.13	1.18	1.35
Water	%	81.6	84.4	85.8
Total organic solids (TOS)[b]	%	17.27	14.42	12.85
Bacillolysin/TOS ratio	UHb/mg TOS	11.141	12.490	14.140
Subtilisin/TOS ratio	UHb/mg TOS	4.366	5.194	5.953

[a]Unit of protease (UHb)/g.
[b]TOS calculated as 100% – % water – % ash.

3.3.3 | Purity

The lead content in the three batches was below 0.03 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).[23] No antimicrobial activity was detected in any of the tested batches.[24]

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

[14]Technical dossier/p. 24/Annex 8.
[15]Technical dossier/p. 24/Annex 8.
[16]Technical dossier/p. 24/Annex 7.
[17]Technical dossier/p. 29.
[18]Technical dossier/p. 27/Annex 10.
[19]Technical dossier/pp. 27–29/Annex 11.
[20]Technical dossier/p. 26/Annexes: 9, 12.
[21]Technical dossier/p. 26/Annexes: 9, 12.
[22]Limit of quantification: Pb = 0.03 mg/kg.
[23]Technical dossier/p. 26/Annexes: 9, 12.
[24]Technical dossier/p. 26/Annexes: 9, 12.

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, 2021).

3.4.1 | Allergenicity

The allergenicity assessment considered only the food enzyme and not any carriers or other excipients that may be used in the final formulation.

The potential allergenicity of the food enzyme containing bacillolysin and subtilisin produced with the *B. amyloliquefaciens* strain AR-383 was assessed by comparing their amino acid sequences 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 for bacillolysin, but for subtilisin, 26 matches with peptidases annotated as respiratory allergens, three peptidases described as contact allergens and one peptidase classified as food allergen were found. The matching food allergen was Cuc m 1, a subtilisin-like protease from *Cucumis melo* melon; the matching contact allergens were Tri r 2, an alkaline protease from *Trichophyton rubrum* (Athlete's foot fungus); Tri me 2, a serine protease from *Arthroderma benhamiae* and Tri s, a serine protease from *Trichophyton schoenleinii*.[25]

No information was available on oral and respiratory sensitisation or elicitation reactions of these bacillolysin and subtilisin under assessment.

Trichophyton rubrum, Arthroderma benhamiae and *Trichophyton schoenleinii* are fungi residing on the skin. No allergic reactions to oral ingestion of such fungal allergens are expected. Several studies have shown that adults respiratorily sensitised to a food enzyme may be able to ingest the corresponding allergen without acquiring clinical symptoms of food allergy (Armentia et al., 2009; Cullinan et al., 1997; Poulsen, 2004).

Allergic reactions after consumption of *Cucumis melo* have been reported (Hassan & Venkatesh, 2015; Neeharika & Sunkar, 2021).

The Panel considered that the risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded, particularly for individuals sensitised to melon, but would not exceed the risk of consuming melon.

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.[26,27]

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	7.92–**15.89**
• Production of cereal-based products other than baked[28]	Flour	7.92–**15.89**
• Production of brewed products	Cereals (malted or unmalted)	6.75–**16.88**
• Production of distilled alcohol	Cereals	3.38–6.75
Processing of dairy products		
• Production of flavouring preparations from dairy products	Dairy products	67.51–**337.56**
• Production of modified milk proteins[29]	Milk proteins	67.51–**675.14**

(Continues)

[25]Technical dossier/pp. 32–33/Annex 13.

[26]Technical dossier/p. 37.

[27]Additional data July 2023/Annex public.

[28]Additional data July 2023/Annex public/Answer 4.

[29]Additional data July 2023/Annex public/Answer 7.

TABLE 2 (Continued)

Food manufacturing process[a]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[b]
Processing of plant- and fungal-derived products		
• Production of protein hydrolysates from plants and fungi	Plant and microbial proteins	67.51–**675.14**
Processing of meat and fish products		
• Production of protein hydrolysates from meat and fish proteins	Meat and fish proteins	67.51–**675.14**
Processing of yeast and yeast products	Yeast	67.51–**675.14**

[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.

In the production of baked products and cereal-based products other than baked, the food enzyme is added to flour during the preparation of the dough or batter.[30] The peptidases cleave the peptide bonds in the gluten network, thus reducing the rigidity of the dough. The food enzyme–TOS remains in the final food products (e.g. bread, biscuits, pasta and breakfast cereals).

In the production of brewed products, the food enzyme is added to cereals during the mashing step. The peptidases hydrolyse proteins in the cereals to release free amino nitrogen for the growth of the brewer's yeast during fermentation.[31] In addition, the partial degradation of protein ensures the clarity of beer. The food enzyme–TOS remains in the beer.

In the production of distilled alcohol, the food enzyme is added during the slurry mixing, liquefaction and fermentation steps.[32] The peptidases 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).

In the production of flavouring preparations from dairy products, the food enzyme is added to modify the sensory properties.[33] The food enzyme–TOS remains in these products, which are subsequently used as ingredients to formulate a variety of foods, such as processed cheese, cheese sauce, cheese powder, salad dressing and snack foods. The food enzyme–TOS remains in those foods.

To manufacture protein hydrolysates, the food enzyme is used to treat proteins from milk (e.g. whey proteins and caseins), plants (e.g. soy, wheat and maize), fungal or animal sources.[34] The food enzyme–TOS remains in the final protein hydrolysates, which are subsequently used as ingredients in a variety of foods, including infant formula, follow-on formula and foods for special medical purposes.

In the processing of yeast and yeast products, the food enzyme is added to yeast cultures, yeast extract or yeast cell walls during different stages of the process.[35] The peptidases are used to hydrolyse insoluble proteins, optimising extraction and improving the functional properties of the yeast products. The food enzyme–TOS remains in yeast products.

Based on the data provided on thermostability (see Section 3.3.1) and the downstream processing steps applied in the food manufacturing processes, the Panel expects that the food enzyme is inactivated in all of the food manufacturing processes in which the food enzyme–TOS is not removed.

3.5.2 | Dietary exposure estimation

In accordance with the guidance document (EFSA CEP Panel, 2021), a dietary exposure was calculated for the eight food manufacturing processes where the food enzyme–TOS remains in the final foods.

Chronic exposure to the food enzyme–TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSA CEP Panel, 2021). The estimation involved the selection of relevant food categories and the 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 (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).

[30]Technical dossier/p. 39.
[31]Technical dossier/p. 40.
[32]Technical dossier/p. 43.
[33]Technical dossier/p. 41.
[34]Additional data July 2023/Annex public/Answers 6 and 7.
[35]Technical dossier/p. 48.

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 1.958 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)	0.106–0.847 (12)	0.147–0.584 (15)	0.116–0.243 (19)	0.031–0.157 (21)	0.035–0.106 (22)	0.025–0.102 (23)
Min–max 95th percentile (number of surveys)	0.270–1.958 (11)	0.397–1.454 (14)	0.270–0.539 (19)	0.076–0.435 (20)	0.073–0.302 (22)	0.050–0.242 (22)

3.5.3 | Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the following sources of uncertainties have been considered and are summarised in Table 4.

TABLE 4 Qualitative evaluation of the influence of uncertainties on the dietary exposure estimate.

Sources of uncertainties	**Direction of impact**
Model input data	
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/–
Use of data from food consumption surveys of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Possible national differences in categorisation and classification of food	+/–
Model assumptions and factors	
Food categories chosen for calculation are broader than milk protein hydrolysates and isolates. Also those relevant to milk protein concentrates were included	+
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.

The conservative approach applied to estimate the exposure to the 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 one food manufacturing process 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 a toxicological assessment was considered unnecessary by the Panel, a 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, the Panel concluded that the food enzyme containing bacillolysin and subtilisin activities produced

with the non-genetically modified *Bacillus amyloliquefaciens* strain AR-383 does not give rise to safety concerns under the intended conditions of use.

5 | DOCUMENTATION AS PROVIDED TO EFSA

Application for authorisation of bacillolysin from a strain of *Bacillus amyloliquefaciens* in accordance with Regulation (EC) No 1331/2008. Submitted by AB Enzymes GmbH.
Additional information. July 2023. Submitted by AB Enzymes GmbH.

ABBREVIATIONS

AMR antimicrobial resistance
bw body weight
QPS qualified presumption of safety
TOS total organic solids
WGS whole genome sequence

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

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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.

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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*, Serbia*, Slovenia, Spain
Children	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Republic of North Macedonia*, Serbia*, Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Bosnia and Herzegovina*, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Montenegro*, Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Bosnia and Herzegovina*, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro*, Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden
The elderly[a]	From 65 years of age and older	Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro*, Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden

*Consumption data from these pre-accession countries are included for testing purpose.

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