Adopted: 13 March 2024

DOI: 10.2903/j.efsa.2024.8723

efsa JOURNAL

SCIENTIFIC OPINION

Safety evaluation of the food enzyme subtilisin from the genetically modified *Bacillus licheniformis* strain NZYM-CB

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Abstract

The food enzyme subtilisin (EC 3.4.21.62) is produced with the genetically modified *Bacillus licheniformis* strain NZYM-CB by Novozymes A/S. The genetic modifications do not give rise to safety concerns. The food enzyme is considered free from viable cells of the production organism and its DNA. It is intended to be used in six food manufacturing processes. The dietary exposure to the food enzyme-TOS was estimated to be up to 0.722 mg TOS/kg body weight (bw) per day in European populations. The production strain of the food enzyme fulfils the requirements for the qualified presumption of safety approach to safety assessment. As no other concerns arising from the manufacturing process were identified, the Panel considered that toxicological tests were not required for the assessment of this food enzyme. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and 20 matches were found, including two food allergens (melon and pomegranate). The Panel considered that the risk of allergic reactions by dietary exposure cannot be excluded, particularly in individuals sensitised to melon and pomegranate, but would not exceed the risk from consumption of melon or pomegranate. 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

bacillopeptidase, *Bacillus licheniformis*, EC 3.4.21.62, genetically modified microorganism, 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;
- 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.

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.

An application has been introduced by the applicant "Novozymes A/S" for the authorisation of the food enzyme Subtilisin from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-CB).

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 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

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002, the European Commission requests the European Food Safety Authority to carry out the safety assessment on the following food enzyme: Subtilisin from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-CB), in accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings.

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme subtilisin from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-CB).

[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, 11.03.2011, pp. 15–24.

Additional information was requested from the applicant during the assessment process on 16 November 2021 and received on 16 May 2022 (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

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

Subtilisins catalyse the hydrolysis of peptide bonds of proteins with broad specificity and with preference for large uncharged residues, releasing peptides and amino acids. The enzyme under assessment is intended to be used in six food manufacturing processes as described in the EFSA guidance (EFSA CEP Panel, 2023): (1) processing of cereals and other grains for the production of brewed products; processing of dairy products for the production of (2) modified milk proteins and (3) flavouring preparations; (4) processing of plant- and fungal-derived products for the production of protein hydrolysates; (5) processing of meat and fish products for the production of protein hydrolysates and (6) processing of yeast and yeast products.

3.1 | Source of the food enzyme

The subtilisin is produced with the genetically modified bacterium *Bacillus licheniformis* strain NZYM-CB, which is deposited at the German Collection of Microorganisms and Cell Cultures GmbH (DSMZ, Germany), with the deposit number [redacted].[4] The production strain was identified as *B. licheniformis* by whole genome sequence (WGS) analysis, showing an average nucleotide identity of [redacted].[5]

The species *B. licheniformis* 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 and no concerns arise from the genetic modifications (EFSA, 2007; EFSA BIOHAZ Panel, 2023). The absence of cytotoxic activity was confirmed using VERO cells.[6] The WGS of the production strain was interrogated for the presence of antimicrobial resistance genes, [redacted]. No genes of concern were identified.[7]

3.1.1 | Characteristics of the parental and recipient microorganisms

The parental strain Ca63 [redacted]

[4]Technical dossier/Annex4/Annex A2.
[5]Technical dossier/Annex4/Annex A1.
[6]Technical dossier/Annex4/Annex A6.
[7]Technical dossier/Additional data May 2022/A5, A5.1 and A5.2.

[redacted][8]

3.1.2 | Characteristics of introduced sequences

[redacted].

3.1.3 | Description of the genetic modification process

The purpose of the genetic modification was to enable the production strain to overproduce subtilisin.

[redacted][9][10][11][12].

3.1.4 | Safety aspects of the genetic modification

The production strain *B. licheniformis* NZYM-CB differs from the recipient strain in its capability to overproduce subtilisin. [redacted].

As the production strain was shown to be free of acquired AMR genes, not to be cytotoxic and as the genetic modifications applied did not raise concerns, it was considered to meet the requirements of the QPS approach for safety assessment.

3.2 | Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004,[13] with food safety procedures based on Hazard Analysis and Critical Control Points and in accordance with current good manufacturing practice.[14]

[8]Technical dossier/Annex4/Annex C1 to C13.
[9]Technical dossier/Additional data May 2022/Annex A6 and Block1 – Block3.
[10]Technical dossier/Additional data May 2022/Updated Annexes C1 and C13 and Block2.
[11]Technical dossier/Additional data May 2022/Block3.
[12]Technical dossier/Additional data May 2022/Annex A6.
[13]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.
[14]Technical dossier/p. 45 and Annex 5.

The production strain is grown as a pure culture using a typical industrial medium in a submerged, fed-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, leaving a supernatant containing the food enzyme. 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.[15] 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.[16]

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 subtilisin is a single polypeptide chain of [redacted] amino acids.[17] The molecular mass of the mature protein, calculated from the amino acid sequence, is [redacted] kDa.[18] The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. A consistent protein pattern was observed across all batches. The gels showed a major protein band corresponding to an apparent molecular mass of about 30 kDa, consistent with the expected mass of the enzyme.[19] The food enzyme was tested for α-amylase, glucoamylase and lipase activities, and none were detected.[20] No other enzymatic activities were reported.

The in-house determination of subtilisin activity is based on the hydrolysis of the *N*-succinyl-Ala-Ala-Pro-Phe *p*-nitroanilide (reaction conditions: pH 9.0, 37°C, 63 s) and determined by measuring the release of *p*-nitroanilide spectrophotometrically at 405 nm. The subtilisin activity is quantified relative to an internal enzyme standard and expressed in Anson Units (Alcalase)/g (AU-A/g).[21]

The food enzyme has a temperature optimum around 70°C (pH 7.0) and a pH optimum around pH 9.0 (30°C). Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures (pH 7.0). The enzyme activity decreased above 65°C, showing no residual activity after pre-incubation above 80°C.[22]

3.3.2 | Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (Table 1).[23] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation was 12.2% and the mean enzyme activity/TOS ratio was 0.04 AU-A/mg TOS.

TABLE 1 Composition of the food enzyme.

		Batches		
Parameters	**Unit**	**1**	**2**	**3**
Subtilisin activity	AU-A/g[a]	4.91	5.20	5.46
Protein	%	11.6	11.9	12.1
Ash	%	0.5	0.5	0.5
Water	%	88.0	87.0	87.0
Total organic solids (TOS)[b]	%	11.5	12.5	12.5
Activity/TOS ratio	AU-A/mg TOS	0.04	0.04	0.04

[a]AU-A/g: Anson Unit (Alcalase)/g (see Section 3.3.1).
[b]TOS calculated as 100% – % water – % ash.

[15]Technical dossier/pp. 45–51.
[16]Technical dossier/Annexes 2.05 and 6.
[17]Technical dossier/p. 32 an Annex 1.
[18]Technical dossier/p. 32 an Annex 1.
[19]Technical dossier/pp. 34.
[20]Technical dossier/pp. 38–39 and Annexes 3.02, 3.03, 3.04.
[21]Technical dossier/pp. 36–37 and Annex 3.01.
[22]Technical dossier/pp. 37–38 and Annex 8.
[23]Technical dossier/p. 33 and Annexes 2, 3, and 9.

3.3.3 | Purity

The lead content in the three commercial batches was below 0.5 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). In addition, the concentrations of arsenic, mercury and cadmium were below the limits of detection (LoD) of the employed methods.[24,25]

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).[26] No antimicrobial activity was detected in any of the tested batches.[27]

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

3.3.4 | Viable cells and DNA of the production strain

The absence of viable cells of the production strain in the food enzyme was demonstrated [redacted].[28]

The absence of recombinant DNA in the food enzyme was demonstrated [redacted].[29]

3.4 | Toxicological data

As the production strain qualifies for the QPS approach of safety assessment and as 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 assessment of allergenicity were necessary.

3.4.1 | Allergenicity

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

The potential allergenicity of the subtilisin produced with the *B. licheniformis* strain NZYM-CB 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, 17 matches with proteases annotated as respiratory allergens, two proteases classified as food allergens and one protease classified as contact allergen were found. The matching food allergens were Cuc m 1, a subtilisin-like protease from *Cucumis melo* (melon) and Pun g 14, a chitinase III from *Punica granatum* (Pomegranate). The matching contact allergen was Tri r 2, an alkaline protease from *Trichophyton rubrum* (Athlete's foot fungus).[30]

No information was available on oral and respiratory sensitisation or elicitation reactions of this subtilisin.

No allergic reactions to oral ingestion of the contact allergen alkaline protease from *Trichophyton rubrum*, a fungus residing on the skin, 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 (Cullinan et al., 1997; Poulsen, 2004). Melon and pomegranate are food allergens.

[redacted], a product that may cause allergies or intolerances (listed in the Regulation (EU) No 1169/2011[31]), is used as raw material. However, during the fermentation process, this product 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 this source are not expected to be present in the food enzyme.

[24]Technical dossier/p. 34 and Annexes 2.04 and 9.

[25]LoDs: Pb = 0.5 mg/kg, As = 0.3 mg/kg, Cd, Hg = 0.05 mg/kg each.

[26]Technical dossier/pp. 35–36 and Annexes 2.07, 2.08, 2.09, 2.10 and 9.

[27]Technical dossier/pp. 33 and Annexes 2.06 and 9.

[28]Technical dossier/Annex4/Annex D1.

[29]Technical dossier/Annex4/Annex D2 and Additional data May 2022.

[30]Technical dossier/pp. 60–64 and Annexes: 7.01 and 7.02.

[31]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.

The Panel considered that the risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded, particularly in individuals sensitised to melon and pomegranate, but would not exceed the risk from consumption of melon or pomegranate.

3.5 | Dietary exposure

3.5.1 | Intended use of the food enzyme

The food enzyme is intended to be used in six food manufacturing processes at the recommended use levels summarised in Table 2.

TABLE 2 Intended uses and recommended use levels of the food enzyme as provided by the applicant.[d]

Food manufacturing process[a]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[b,c]	
Processing of cereals and other grains			
• Production of brewed products	Cereals (malted or unmalted)	12.5–**150**	
Processing of dairy products			
• Production of modified milk proteins	Whey protein concentrate	5–**250**	
• Production of flavouring preparations from dairy products	Cheese, milk powder	1.2–**150**	
Processing of plant- and fungal-derived products			
• Production of protein hydrolysates from plants and fungi	Protein concentrates or isolates from plants	5–250	Protein supplements
		5–**625**	Other products
Processing of meat and fish products			
• Production of protein hydrolysates from meat and fish proteins	Protein concentrates or isolates from meat and fish	5–250	Protein supplements
		5–**625**	Other products
Processing of yeast and yeast products	Yeast	50–**200**	

[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]Based on the mean activity/mg TOS of 0.04 AU-A/mg TOS.

[c]The numbers in bold represent the maximum recommended use levels, which were used for calculation.

[d]Technical dossier/Additional data May 2022/Answers add.info_2021-00295_Subtilisin from NZYM-CB - Annex.

In the production of brewed products, the food enzyme is added to malted or unmalted grains during the mashing step.[32] The subtilisin hydrolyses proteins in the cereals to release free amino nitrogen for the optimal growth of the Brewer's yeast during fermentation.[33] The food enzyme-TOS remains in the beers.

In the production of modified milk proteins, the food enzyme is added to whey protein concentrate[34] to achieve the desired degree of hydrolysis. The resulting whey protein hydrolysates are subsequently used as ingredients in a variety of foods, including protein and amino acids supplements.[35] The food enzyme-TOS remains in the final foods.

In the production of flavouring preparations from dairy products, the food enzyme is added to cheese to modify the sensory properties.[36] The food enzyme-TOS remains in these enzyme-modified cheeses, which are subsequently used as an ingredient in the formulation of a variety of foods, such as processed cheese, cheese sauce, cheese powder, salad dressing and snack foods.

In the production of hydrolysed proteins from different sources, the food enzyme is added to the protein isolated from plant or animal sources during the hydrolysis step.[37] The subtilisin is used alone or together with other peptidases to achieve the desired degree of hydrolysis, to improve protein solubility and to enhance flavours.[38] The food enzyme-TOS remains in these protein hydrolysates.

In the processing of yeast and yeast products, the food enzyme is added during the autolysis step.[39] The subtilisin hydrolyses insoluble proteins, optimising the extraction process and improving the sensory properties of the yeast extract,[40] which are used (in paste or powder form) as an ingredient in a wide range of foods. The food enzyme-TOS remains in yeast extracts.

[32]Technical dossier/p. 78.

[33]Technical dossier/p. 77.

[34]Technical dossier/Additional data May 2022/Answers add.info_2021-00295_Subtilisin from NZYM-CB - Annex.

[35]Additional data May 2022/Answers add.info_2021-00295_Subtilisin from NZYM-CB - Annex.

[36]Additional data May 2022/Answers add.info_2021-00295_Subtilisin from NZYM-CB - Annex.

[37]Technical dossier/p. 75.

[38]Additional data May 2022/Answers add.info_2021-00295_Subtilisin from NZYM-CB - Annex.

[39]Additional data May 2022/Answers add.info_2021-00295_Subtilisin from NZYM-CB - Annex.

[40]Technical dossier/p. 76.

Based on the data provided on thermostability (see Section 3.3.1) and the downstream processing step applied in the food manufacturing processes, it was expected that the enzyme is inactivated in all the food manufacturing processes listed in Table 2.

3.5.2 | Dietary exposure estimation

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 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 0.722 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)	0.071–0.333 (12)	0.069–0.218 (15)	0.041–0.130 (19)	0.011–0.081 (21)	0.027–0.183 (22)	0.009–0.101 (23)
Min–max 95th percentile (number of surveys)	0.171–0.722 (11)	0.205–0.553 (14)	0.150–0.392 (19)	0.034–0.312 (20)	0.112–0.757 (22)	0.049–0.371 (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	
Selection of broad FoodEx categories for the exposure assessment	+
Exposure to food enzyme–TOS always calculated based on the recommended maximum use level	+
The calculation for the production of modified milk proteins included not only food groups containing whey protein hydrolysates but also those containing milk protein isolates and concentrates	+
For yeast processing, although only yeast extract is indicated as the final food ingredient,[a] the food categories chosen for calculation cover also those containing mannoproteins resulting from the treatment of yeast cell walls	+
In the production of protein hydrolysates from different sources, exposure to protein supplements was calculated using the TOS indicated for the other products	+
Use of recipe fractions to disaggregate FoodEx categories	+/–
Use of technical factors in the exposure model	+/–

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

[a]Technical dossier/Additional data May 2022/Answers add.info_2021-00295_Subtilisin from NZYM-CB - Annex.

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.

3.6 | Margin of exposure

Since no toxicological assessment was considered necessary 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 other concerns, the Panel concluded that the food enzyme subtilisin produced with the genetically modified *Bacillus licheniformis* strain NZYM-CB does not give rise to safety concerns under the intended conditions of use.

The CEP Panel considered the food enzyme free from viable cells of the production organism and its recombinant DNA.

5 | DOCUMENTATION AS PROVIDED TO EFSA

Application for authorisation of subtilisin produced by a genetically modified strain of *Bacillus licheniformis* (strain NZYM CB). September 2021. Submitted by Novozymes A/S.

Additional data. May 2022. Submitted by Novozymes A/S.

ABBREVIATIONS

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
LoD	limit of detection
TOS	total organic solids
WHO	World Health Organisation

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-2021-00295

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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., Aguilera, J., Andryszkiewicz, M., … Chesson, A. (2024). Safety evaluation of the food enzyme subtilisin from the genetically modified *Bacillus licheniformis* strain NZYM-CB. *EFSA Journal*, *22*(4), e8723. https://doi.org/10.2903/j.efsa.2024.8723

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 not reported in Table 3 of this opinion; however, they are included in Appendix B for testing purpose.

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