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SCIENTIFIC OPINION

Safety evaluation of the food enzyme β-galactosidase from the genetically modified *Bacillus licheniformis* strain DSM 34099

EFSA panel on Food Enzymes (FEZ) | Holger Zorn | José Manuel Barat Baviera | Claudia Bolognesi | Francesco Catania | Gabriele Gadermaier | Ralf Greiner | Baltasar Mayo | Alicja Mortensen | Yrjö Henrik Roos | Marize L. M. Solano | Monika Sramkova | Henk Van Loveren | Laurence Vernis | Yrjö Roos | Magdalena Andryszkiewicz | Daniele Cavanna | Natalia Kovalkovicova | Silvia Peluso | Rita Ferreira de Sousa

Correspondence: fip@efsa.europa.eu

Abstract

The food enzyme β-galactosidase (β-D-galactoside galactohydrolase; EC 3.2.1.23) is produced with the genetically modified *Bacillus licheniformis* strain DSM 34099 by Kerry Group Services International, Ltd. (KGSI). The genetic modifications do not give rise to safety concerns. The food enzyme is free from viable cells of the production organism and its DNA. The production strain met the requirements for the qualified presumption of safety (QPS) approach. The food enzyme is intended to be used in two food manufacturing processes. Dietary exposure was estimated to be up to 7.263 mg total organic solids/kg body weight per day in European populations. Given the QPS status of the production strain and the absence of concerns resulting from the food enzyme manufacturing process, toxicity tests, other than an assessment of allergenicity, were considered unnecessary by the Panel. A search for the identity of the amino acid sequence of the food enzyme to known allergens was made and one match with a food allergen from kiwi fruit was found. The Panel considered that a risk of allergic reactions upon dietary exposure to this food enzyme, particularly in individuals sensitised to kiwi fruit, cannot be excluded. 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

Bacillus licheniformis, EC 3.2.1.23, EFSA-Q-2023-00443, food enzyme, genetically modified microorganism, lactase, β-galactosidase

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 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 1332/2008 on food enzymes.

On 27 April 2023, a new application has been introduced by the applicant "Chr. Hansen A/S" for the authorisation of the food enzyme Beta-galactosidase from a genetically modified *Bacillus licheniformis* (strain DSM 34099).

1.1.2 | Terms of Reference

The European Commission requests the European Food Safety Authority (EFSA) to carry out the safety assessment and the assessment of possible confidentiality requests of the following food enzyme: Beta-galactosidase from a genetically modified *Bacillus licheniformis* (strain DSM 34099), in accordance with Regulation (EC) No 1331/2008 establishing a common authorization procedure for food additives, food enzymes and food flavourings.

1.1.3 | Interpretation of Terms of Reference

The application was submitted initially by Chr. Hansen A/S under Article 7(2) of Regulation No 1332/2008. On 16 May 2024, Chr. Hansen A/S notified the Commission of the transfer of ownership of the Application FEN 2023–15,535 (EFSA-Q-2023-00443) to the Kerry Group P.L.C. and updated the applicant as 'Kerry Group Services International, Ltd. (KGSI)'. This scientific opinion reports both the former and the current applicant for transparency.

[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.

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme Beta-galactosidase from a genetically modified *Bacillus licheniformis* (strain DSM 34099).

Additional information was requested from the applicant during the assessment process on 19 April 2024 and received on 10 May 2024 (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 guidance documents of the EFSA Scientific Committee.

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

2.3 | Public consultation

According to Article 32c(2) of Regulation (EC) No 178/2002[3] and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations, EFSA carried out a public consultation on the non-confidential version of the technical dossier from 8 May to 29 May 2024.[4] No comments were received.

3 | ASSESSMENT

IUBMB nomenclature	β-Galactosidase
Systematic name	β-D-galactoside galactohydrolase
Synonyms	Lactase, β-D-lactosidase
IUBMB no	3.2.1.23
CAS no	9031-11-2
EINECS no	232-864-1

β-Galactosidases catalyse the hydrolysis of terminal non-reducing β-D-galactose residues in β-D-galactosides. The food enzyme under assessment is intended to be used in two food manufacturing processes as described in the EFSA guidance (EFSA CEP Panel, 2023): processing of dairy products for the production of (1) lactose reduced dairy products and (2) fermented dairy products.

3.1 | Source of the food enzyme

The β-galactosidase is produced with the genetically modified bacterium *Bacillus licheniformis* strain DSM 34099, which is deposited at the German Collection of Microorganisms and Cell Cultures GmbH (Germany) with the deposit number DSM [redacted].[5] The production strain was identified as *Bacillus licheniformis* by whole genome sequencing analysis [redacted].[6]

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 the genetic modifications do not raise any concerns.[7] A cytotoxicity test made with supernatants indicated that the production strain *B. licheniformis* strain DSM 34099 did not induce cell damage to VERO cells

[3]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

[4]Accesible at: https://connect.efsa.europa.eu/RM/s/consultations/publicconsultation2/a0lTk000000olAD/pc0932.

[5]Technical dossier/Risk assessment/Annex 1.

[6]Technical dossier/Risk assessment/EZBBIDPS_identity_confidential.

[7]https://zenodo.org/records/7554079.

using the lactate dehydrogenase assay.[8] WGS analysis of the production strain against two regularly updated databases using 80% identity and 70% coverage did not identify antimicrobial resistance genes of concern.[9]

3.1.1 | Characteristics of the parental and recipient microorganisms

The parental microorganism is the bacterium [redacted].[10]

The genetically modified recipient strain was developed from the parental strain by subsequent steps [redacted].[11]

3.1.2 | Characteristics of introduced sequences

The sequence encoding the β-galactosidase [redacted].[12] [redacted].[13]

[redacted].

3.1.3 | Description of the genetic modification

The purpose of the genetic modification was to enable the production strain to synthesise β-galactosidase. For this purpose, the recipient strain was [redacted].[14]

3.1.4 | Safety aspects of the genetic modification

The technical dossier contains all necessary information on the recipient microorganism, the donor organism and the genetic modification process.

The production strain *Bacillus licheniformis* DSM 34099 differs from the recipient strain in its capacity to produce β-galactosidase and its ability [redacted].

No issues of concern arising from the genetic modifications were identified by the Panel. As the other qualifications have been met, the production strain is 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,[15] with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current good manufacturing practice.[16]

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, [redacted] and the solid

[8]Technical dossier/Risk assessment/A3739_cytotoxicity_confidential.

[9]Technical dossier/Risk assessment/EZZBB01: Source of the food enzyme.

[10]Technical dossier/Risk assessment/22AN4823: DNA extraction and genome sequencing.

[11]Technical dossier/Risk assessment/EZBBMS: Characterisation of the genetic modifications of production strain DSM 34099 using WGS.

[12]Technical dossier/Risk assessment/EZBBMS_Characteristics_confidential.

[13]Technical dossier/Risk assessment/EXBBMS_Characteristics_Confidential.

[14]Technical dossier/Risk assessment/EZBBMS_Characteristics_confidential/EZBB01_Source of the food enzyme & EZBBMS: Characterisation of the genetic modifications of production strain DSM 34099 using WGS.

[15]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.

[16]Technical Dossier/Risk Assessment/Manufacturing process of the Food enzyme/EZBB02.Manufacturing process of the food enzyme; ISO 22000.

biomass is removed from the fermentation broth by filtration. The filtrate containing the enzyme is 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.[17] 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.[18]

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 β-galactosidase is a single polypeptide chain of [redacted] amino acids.[19] The molecular mass of the mature protein, calculated from the amino acid sequence, is 125.0 kDa.[20] The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.[21] A consistent protein pattern was observed across all batches. The gel showed a major protein band corresponding to an apparent molecular mass of about 125 kDa, consistent with the expected mass of the enzyme. No other enzyme activities were reported.

The applicant's in-house determination of β-galactosidase activity is based on the hydrolysis of *o*-nitrophenyl-β-D-galactopyranoside (reaction conditions: pH 6.5, 30°C, 10 min) and determined by measuring the release of *o*-nitrophenol spectrophotometrically. The enzyme activity is expressed in BLU lactase units (BLU)/g. One BLU is defined as the amount of enzyme that produces [redacted] of *o*-nitrophenol per minute under the conditions of the assay.[22]

The food enzyme has a temperature optimum around 60°C (pH 6.5) and a pH optimum around pH 6.3 (30°C).[23] Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures (pH 6.5). Enzyme activity decreased above 35°C showing no residual activity above 45°C.[24]

3.3.2 | Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (Table 1).[25] The mean total organic solids (TOS) of the three batches for commercialisation was 9.2% and the mean enzyme activity/TOS ratio was 1479 BLU/mg TOS.

TABLE 1 Composition of the food enzyme.

Parameters	Unit	Batches		
		1	2	3
β-Galactosidase activity	BLU/g[a]	128,802	135,080	146,198
Protein	%	7.2	7.7	8.0
Ash	%	1.5	1.4	1.6
Water	%	89.7	89.2	88.9
Total organic solids (TOS)[b]	%	8.8	9.4	9.5
Activity/TOS ratio	BLU/mg TOS	1462	1437	1539

[a]BLU Lactase Activity (see Section 3.3.1).

[b]TOS calculated as 100% – % water – % ash.

[17]Technical Dossier/Risk Assessment/Manufacturing process of the food enzyme/EZBB02.Manufacturing process of the food enzyme.

[18]Technical Dossier/Risk Assessment/Manufacturing process of the food enzyme/EZBB02.Manufacturing process of the food enzyme; EZBBRMAF_antifoam; EZBBRMFL_flocculant; EZBBRM_List of raw materials.

[19]Technical Dossier/Risk Assessment/Chemical composition, properties and purity of the food enzyme/EZBB03.Chemical composition properties and purity of the food enzyme, p. 3.

[20]Technical Dossier/Risk Assessment/Chemical composition, properties and purity of the food enzyme/EZBB03, p. 3.

[21]Technical Dossier/Risk Assessment/Chemical composition, properties and purity of the food enzyme/EZBB03, p. 3; EZBBSPRB3_Protein pattern characteristics.

[22]Technical Dossier/Risk Assessment/Chemical composition, properties and purity of the food enzyme/EZBB03, p. 4; EZBATRB3_Enzyme activity measurement; CoA_220812F6-Reg_activity; CoA_220812F7-Reg_activity; CoA_220826F6-Reg_activity.

[23]Technical Dossier/Risk Assessment/Chemical composition, properties and purity of the food enzyme/EZBB03, p. 7; EZBBPHB2_pH profile.

[24]Technical Dossier/Risk Assessment/Chemical composition, properties and purity of the food enzyme/EZBB03, p. 5; EZBBTRTSB2_Temp_profile_stability.

[25]Technical Dossier/Risk Assessment/Chemical composition, properties and purity of the food enzyme/EZBB03, p. 7; CoA_220812F6-Reg_actvity; CoA_220812F6-Reg_Chem_AM; CoA_220812F7-Reg_activity; CoA220812F7-Reg_Chem_AM; CoA_220826F6-Reg_Activity; CoA_220826F6-Reg_Chem_AM.

3.3.3 | Purity

The lead content in the three commercial batches was below 0.05 mg/kg[26,27] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006, Volume 4).

The food enzyme complies with the microbiological criteria for total coliforms and *Escherichia coli* and no antimicrobial activity was detected in any of the tested batches as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006, Volume 4).[28]

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

3.3.4 | Viable cells and DNA of the production strain

The absence of viable cells of the production strain in the food enzyme was demonstrated in three independent batches analysed in triplicate. Ten millilitres for each sample was incubated in a non-selective agar medium at 37°C for 60–70 h. No colonies were produced. A positive control was included.[29]

The absence of recombinant DNA in the food enzyme was demonstrated by polymerase chain reaction (PCR) analysis of three batches in triplicate. No DNA was detected with primers that would amplify an 873-bp fragment that includes the *lacZ-BB* gene, with a limit of detection of 10 ng spiked DNA/g food enzyme.[30]

3.4 | Toxicological data

As the production strain qualifies for the QPS approach of safety assessment and no issues of concern arising from the production 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 carriers or other excipients that may be used in the final formulation.

The potential allergenicity of the β-galactosidase produced with the *Bacillus licheniformis* strain DSM 34099 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, one match was found.[31] The matching allergen actinidin (Act d 1), produced by *Actinidia deliciosa* (kiwi fruit), is a known food allergen.

No information is available on oral and respiratory sensitisation or elicitation reactions of this β-galactosidase.

Kiwi fruit allergy is increasingly being reported, and it can either be ascribed to cross-reaction to birch pollen and latex or to a primary kiwi allergy (Le et al., 2013).

Cases of occupational allergy following exposure by inhalation of β-galactosidase have been reported (Bernstein et al., 1999; Muir et al., 1997; Stöcker et al., 2016). Several studies have shown that adults sensitised to respiratory allergens may ingest the allergenic enzymes without acquiring clinical symptoms of food allergy (Armentia et al., 2009; Brisman, 2002; Cullinan et al., 1997; Poulsen, 2004). Two case reports describing allergic reactions (swollen throat, shortness of breath and difficulty in swallowing) following ingestion of lactase pills have been reported (Binkley, 1996; Voisin & Borici-Mazi, 2016).

[redacted], a known source of allergens, is present in the media fed to microorganisms. However, during the fermentation process, the extract will be degraded and used by microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the microbial biomass and fermentation solids are removed. The Panel considered that no potentially allergenic residues from this source are present in the food enzyme.

The Panel considered that a risk of allergic reactions upon dietary exposure to this food enzyme, particularly in individuals sensitised to kiwi fruit, cannot be excluded.

[26]Technical Dossier/Risk Assessment/Chemical composition, properties and purity of the food enzyme/EZBB03, p. 9; EZBBHGRB3_lead.

[27]LoQ: Pb = 0.05 mg/kg.

[28]Technical Dossier/Risk Assessment/Chemical composition, properties and purity of the food enzyme/EZBB03, p. 9; EZBBECETRB3; CoA_microbial_3-batches.

[29]Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/CoA_absence_viable_cells_3_batches_confidential.

[30]Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/CoA_absence_DNA_3_batches_confidential.

[31]Technical dossier/Allergenicity/EZBBDALS_allergenicity.

3.5 | Dietary exposure

3.5.1 | Intended use of the food enzyme

The food enzyme is intended to be used in two 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.[32]

Food manufacturing process[a]	Raw material (RM)	Recommended use level (mg TOS/litre RM)[b]
Processing of dairy products		
• Production of lactose-reduced dairy products	Milk, whey	13.5–20.3 for cheese
		67.6–**101.4** for drinking milk, dairy powder, ice-cream, dairy deserts and whey syrups
• Production of fermented dairy products	Milk	13.5–**20.3**

[a]The name has been harmonised by EFSA in accordance with the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023).

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

In all the food manufacturing processes involved, β-galactosidase hydrolyses lactose to release glucose and galactose. The treatment makes dairy products more suitable for lactose-intolerant individuals and sweeter.[33]

In the production of lactose-reduced dairy products, the food enzyme can be added to milk, whey or demineralised whey before or after the thermal treatment.[34] In the production of milk powders, the enzyme is added to milk before evaporation and spray drying. The enzymatically treated milk can be consumed directly, but can also be used as ingredient in a variety of foods.[35] The food enzyme-TOS remain in the final foods.

In the production of dairy desserts and ice creams, this β-galactosidase can be also added during the filling[36] or during the ageing steps.[37] The enzymatic treatment also prevents the sandiness caused by lactose crystallisation in frozen desser-ts such as ice cream.[38] The food enzyme-TOS remain in the final foods.

In the production of fermented dairy products, the food enzyme is added to milk before the pasteurisation or at the beginning of the fermentation process.[39] The food enzyme-TOS remain in the final foods (e.g. lactose-reduced yoghurts and similar products).[40]

Based on data provided on thermostability (see Section 3.3.1), it is expected that this β-galactosidase will be inactivated in many of the final foods. When added after any thermal treatment, such as the production of UHT milk, the enzyme will remain in its active form.

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 7.263 TOS/kg body weight (bw) per day in toddlers at the 95th percentile.

[32]Technical Dossier/ Risk Assessment/ EZBB09: Intended uses in food and use levels/Table 2.
[33]Technical Dossier/Risk Assessment/EZBB09: Intended uses in food and use levels/p. 3.
[34]Technical Dossier/Risk Assessment/EZBB09: Intended uses in food and use levels/Figures 1–2.
[35]Technical Dossier/Risk Assessment/EZBB09: Intended uses in food and use levels/Figures 4–5.
[36]Technical Dossier/Risk Assessment/EZBB09: Intended uses in food and use levels/Figure 7.
[37]Technical Dossier/Risk Assessment/EZBB09: Intended uses in food and use levels/Figure 6.
[38]Technical Dossier/Risk Assessment/EZBB09: Intended uses in food and use levels/p. 10.
[39]Technical Dossier/Risk Assessment/EZBB09: Intended uses in food and use levels/Figure 3.
[40]Technical Dossier/Risk Assessment/EZBB09: Intended uses in food and use levels/Figure 8.

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)	0.099–1.580 (12)	0.084–2.935 (15)	0.572–2.724 (19)	0.037–0.993 (21)	0.047–0.370 (22)	0.021–0.339 (23)
Min–max 95th percentile (number of surveys)	0.270–4.766 (11)	2.221–7.263 (14)	1.595–4.725 (19)	0.122–2.116 (20)	0.140–1.184 (22)	0.337–0.855 (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	+
Exposure from production of lactose-reduced dairy products, including cheese, was calculated using the TOS indicated for drinking milk and 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.

The conservative approach applied to estimate the dietary 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 issues of concern arising from the food enzyme production process, the Panel concluded that the food enzyme β-galactosidase produced with the genetically modified *Bacillus licheniformis* strain DSM 34099 does not give rise to safety concerns under the intended conditions of use.

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

5 | DOCUMENTATION AS PROVIDED TO EFSA

Beta-galactosidase expressed in *Bacillus licheniformis* DSM 34099. March 2023. Submitted by Chr. Hansen A/S.

Additional information. May 2024. Submitted by Chr. Hansen A/S.

ABBREVIATIONS

bw body weight
CAS Chemical Abstracts Service
CEP EFSA Panel on Food Contact Materials, Enzymes and Processing Aids

EC	European Commission
EINECS	European Inventory of Existing Commercial Chemical Substances
EU	European Union
FAO	Food and Agricultural Organisation of the United Nations
FEZ	EFSA panel on Food Enzymes
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
MOE	margin of exposure
PCR	polymerase chain reaction
QPS	qualified presumption of safety
RM	raw material
TOS	total organic solids
WGS	whole genome sequencing
WHO	World Health Organization

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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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PANEL MEMBERS
José Manuel Barat Baviera, Claudia Bolognesi, Francesco Catania, Gabriele Gadermaier, Ralf Greiner, Baltasar Mayo, Alicja Mortensen, Yrjö Henrik Roos, Marize de Lourdes Marzo Solano, Monika Sramkova, Henk Van Loveren, Laurence Vernis, and Holger Zorn.

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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 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).