SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 23 March 2021

doi: 10.2903/j.efsa.2021.6562

Safety evaluation of the food enzyme endo-1,4-β-xylanase from the genetically modified *Bacillus subtilis* strain DP-Ezd31

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), Claude Lambré, José Manuel Barat Baviera, Claudia Bolognesi, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis, Holger Zorn, Magdalena Andryszkiewicz, Giovanni Bernasconi, Ana Gomes, Natalia Kovalkovicova, Yi Liu and Andrew Chesson

Abstract

The food enzyme endo-1,4-β-xylanase (4-β-D-xylan xylanohydrolase; EC 3.2.1.8) is produced with a genetically modified *Bacillus subtilis* strain DP-Ezd31 by Danisco US Inc. The production strain of the food enzyme contains multiple copies of a known antimicrobial resistance gene. However, based on the absence of viable cells and DNA from the production organism in the food enzyme, this is not considered to be a safety concern. The production strain was not shown to meet the criteria for Qualified Presumption of Safety (QPS) approach to safety assessment. The substitute studies provided were not considered suitable for the toxicological assessment of this food enzyme. A search for similarity of the amino acid sequence to known allergens was made and no match was found. The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but the likelihood for this to occur is considered to be low. In the absence of suitable toxicological studies, the Panel cannot conclude on the safety of the food enzyme.

Keywords: Food enzyme, EC 3.2.1.8, endo-1, 4-β-xylanase, xylanase, *Bacillus subtilis*, genetically modified microorganism

Requestor: European Commission

Question number: EFSA-Q-2015-00839

Correspondence: fip@efsa.europa.eu

Panel members: José Manuel Barat Baviera, Claudia Bolognesi, Andrew Chesson, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Claude Lambré, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Vittorio Silano (until 21 December 2020 †), Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis and Holger Zorn.

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.

Declarations of interest: The declarations of interest of all scientific experts active in EFSA's work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

Suggested citation: EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré C, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mengelers M, Mortensen A, Rivière G, Steffensen I-L, Tlustos C, Van Loveren H, Vernis L, Zorn H, Andryszkiewicz M, Bernasconi G, Gomes A, Kovalkovicova N, Liu Y and Chesson A, 2021. Scientific Opinion on the safety evaluation of the food enzyme endo-1,4-β-xylanase from the genetically modified *Bacillus subtilis* strain DP-Ezd31. EFSA Journal 2021;19(4):6562, 15 pp. https://doi.org/10.2903/j.efsa.2021.6562

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.

Table of contents

1. Introduction

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

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

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

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

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

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

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

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

1.1.1. Background as provided by the European Commission

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

Five applications have been introduced for the authorisation of 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™).

Following the requirements of Article 12.1 of Commission Regulation (EU) No 234/2011[3] implementing Regulation (EC) No 1331/2008[4], 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] 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, p. 15–24.

[4] 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, p. 1–6.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb44), Bacillolysin from *Bacillus amyloliquefaciens*, Beta-galactosidase from a genetically modified strain of *Bacillus subtilis* (DP-Ezg29), Endo-1,4-beta-xylanase from a genetically modified strain of *Bacillus subtilis* (Dp-Ezd31) and Beta-Fructofuranosidase from *Aspergillus fijiensis* (strain ATCC® 20611™) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. 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 endo-1,4-β-xylanase from a genetically modified *B. subtilis* (strain DP-Ezd31).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme endo-1,4-β-xylanase from a genetically modified *B. subtilis* (strain DP-Ezd31).

Additional information was requested from the applicant during the assessment process on 7 February 2019 and 12 December 2019, and was consequently provided (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, 2009b) as well as in the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) and following the relevant existing guidances of EFSA Scientific Committees.

The current 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) has been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance with the methodology described in the 'CEF Panel statement on the exposure assessment of food enzymes' (EFSA CEF Panel, 2016).

3. Assessment

IUBMB nomenclature:	Endo-1,4-β-xylanase
Systematic name:	4-β-D-xylan xylanohydrolase
Synonyms:	xylanase; endo-1,4-D-β-xylanase
IUBMB No:	3.2.1.8
CAS No:	9025-57-4
EINECS No:	232-800-2

The endo-1,4-β-xylanase catalyses the random hydrolysis of 1,4-ß-D-xylose linkages in xylans (including arabinoxylans) resulting in the generation of (1→4)-β-D-xylan oligosaccharides of different lengths. It is intended to be used in baking processes and grain treatment for the production of starch and gluten fractions.

3.1. Source of the food enzyme

The endo-1,4-β xylanase is produced with a genetically modified bacterium *B. subtilis* strain DP-Ezd31[5], which is deposited at the collection of Westerdijk Fungal Biodiversity Institute (The Netherlands) with deposition number [redacted][6].

The species *B. subtilis* 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 genes

[5] Additional information November 2019/Annex AC.
[6] Additional information November 2019/Annex AD.

and toxigenic activity are verified for the specific strain used. No cytotoxicity studies were provided for the production strain. Therefore, the production strain cannot be considered to qualify for the QPS status.

3.1.1. Characteristics of the parental and recipient microorganisms

The parental microorganism [redacted][7][redacted][8]

Strain [redacted] is an intermediate [redacted][9] The recipient strain [redacted][10] [redacted][11] [redacted]

A plasmid [redacted][12] [redacted]

3.1.2. Characteristics of introduced sequences

The sequence encoding the endo-1,4-β-xylanase [redacted]

3.1.3. Description of the genetic modification process

The purpose of genetic modification was to enable the production strain to synthesise endo-1,4-β-xylanase [redacted][10]

[7] Technical dossier/1st submission/Annex V; Additional information November 2019.
[8] Additional information November 2019/Annex AM.
[9] Additional information November 2019/Annex AF.
[10] Technical dossier/2nd submission/Annex S.
[11] Additional information November 2019/Annex AH.
[12] Additional information November 2019/Annex AG.

Following [redacted][13]

Polymerase chain reaction (PCR) and WGS analysis[11] indicated that [redacted][10]

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 *B. subtilis* DP-Ezd31 ([redacted]) differs from the recipient strain [redacted] in its capacity to produce the endo-1,4-β-xylanase enzyme from [redacted]. The presence of the enzyme encoding gene was confirmed by Southern and WGS analysis.[14]

A [redacted] as indicated by WGS analysis,[11] which is not of concern. WGS analysis of the production strain also revealed a frameshift in the coding sequence of [redacted] and of [redacted]. In the absence of a cytotoxicity study, the possible hazard posed by these modifications cannot be assessed.

The genetic modifications resulted in the presence of multiple copies of [redacted] in the genome of the production strain, which is considered a hazard.

No other concern resulting from the genetic modification was identified.

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 (HACCP), and in accordance with current Good Manufacturing Practice (GMP).

The production strain is grown as a pure culture using a typical industrial medium in a submerged, batch or 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 in which enzyme protein is retained while most of the low molecular weight material passes the filtration membrane and is discarded.[16] 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.[17]

The Panel considered that sufficient information has been provided on the manufacturing process and the quality assurance system implemented by the applicant.

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The endo-1,4-β-xylanase is a single polypeptide chain of [redacted] amino acids.[18] The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be [redacted] kDa.[19] The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis. A consistent protein pattern was observed across all batches. The gel showed a single major

[13] Additional information November 2019/Annex 1 and AE.
[14] Technical dossier/2nd submission/Annex Y.
[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/1st submission/pg. 51-59/Annex L; Additional information November 2019.
[17] Technical dossier/1st submission/pg. 51-59/Annex M; Additional information November 2019/Annex Z and AA.
[18] Technical dossier/1st submission/Annex I.
[19] Technical dossier/1st submission/Section 3.2.1.1.2.3 and Annex I.

protein band corresponding to an apparent molecular mass of about [redacted] kDa, together with other bands of lesser staining intensity.[20] No other enzymatic activities were reported.[21]

The in-house determination of endo-1,4-β-xylanase activity is based on the hydrolysis of azurine-crosslinked wheat arabinoxylan with the released azurine measured spectrophotometrically at 590 nm (pH 5.0, 40°C, 10 min). The enzyme activity is determined in comparison to an internal standard and expressed as Danisco Endo-Xylanase Units (DXU)/g.[22]

The food enzyme has a temperature optimum around 55°C (pH 5.0) and pH optimum around pH 6 (40°C).[23] No enzyme activity was detected at temperatures above 60°C after incubation for 5 min.[21]

3.3.2. Chemical parameters

Data on chemical parameters of the food enzyme were provided for three batches used for commercialisation (Table 1).[24] The average total organic solids (TOS) content of the three food enzyme batches for commercialisation is 5.04% and the average enzyme activity/TOS ratio of is 38,723 DXU/mg TOS.

Table 1: Compositional data of the food enzyme

Parameter	Unit	Batches		
		1	2	3
Endo-1,4-β-xylanase activity	DXU/g batch[(a)]	1,403,058	1,730,000	2,778,345
Protein	%	2.13	2.50	3.65
Ash	%	0.47	0.43	0.04
Water	%	95.46	95.34	93.15
Total organic solids (TOS)[(b)]	%	4.07	4.23	6.81
Endo-1,4-β-xylanase activity/mg TOS	DXU/mg TOS	34,473	40,898	40,798

(a): DXU/g: Danisco Endo-xylanase Units/g (see Section 3.3.1).
(b): TOS calculated as 100% – % water –%.

3.3.3. Purity

The lead content in the three commercial batches was below 5 mg/kg[25,26], which complies with the specification for lead (≤ 5 mg/kg) 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, *E. coli* and *Salmonella*), as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). No antimicrobial activity was detected in any of the tested batches (FAO/WHO, 2006).[26]

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

3.3.4. Viable cells and DNA of the production strain

The absence of the production strain in the food enzyme was demonstrated in nine independent batches of the food enzyme. [redacted] No colonies were produced.[27]

The absence of recombinant DNA in the food enzyme was demonstrated by PCR analysis of three batches in triplicate. No DNA was detected [redacted][28]

[20] Technical dossier/XX submission/Section 3.2.1.1.2.1.
[21] Technical dossier/1st submission/Section 3.2.1.1.2.3.
[22] Technical dossier/XX submission/Annex E.
[23] Technical dossier/1st submission/pg. 42–43; Additional information November 2019/Annex AB.
[24] Technical dossier/1st submission/ Annex D and E.
[25] LOD: Pb = 5 mg/kg.
[26] Technical dossier/1st submission/Annex G and H_updated.
[27] Technical dossier/1st submission/Annex G; Additional data November 2019/Annex AI; Additional data December 2020/Annex AU.
[28] Technical dossier/1st submission/Annex U; Additional data November 2019/Annex AJ; Additional data December 2020/Annex AV.

3.4. Toxicological data

No toxicological studies were provided for the endo-1,4-β-xylanase food enzyme produced with the *B. subtilis* strain DP-Ezd31. Instead, the applicant argued that the assessment of the endo-1,4-β-xylanase produced by *B. subtilis* strain DP-Ezd31 could be based on toxicological data from another food enzyme – endo-1,3(4)-β-glucanase produced with the *B. subtilis* strain DP-Emz28, previously submitted to EFSA (Question No EFSA-Q-2015-00828).

The Panel considered that, due to genetic differences between these two strains, the resulting TOS content is not comparable. As a consequence, the Panel concluded that the provided data are not suitable for safety assessment of the endo-1,4-β-xylanase food enzyme produced with the *B. subtilis* strain DP-Ezd31.

3.4.1. Allergenicity

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

The potential allergenicity of the endo-1,4-β-xylanase produced with the genetically modified *B. subtilis* strain DP-Ezd31 was assessed by comparing its amino acid sequence with those of known allergens according to the scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms (EFSA GMO Panel, 2010). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, no match was found.[29]

No information is available on oral sensitisation or elicitation reactions of this endo-1,4-β-xylanase.

Respiratory allergy, e.g. baker's asthma, following occupational exposure to xylanase has been described in some epidemiological studies (Elms et al., 2003; Martel et al., 2010) and case reports (Merget et al., 2001). However, several studies have shown that adults with occupational asthma to an enzyme may be able to ingest the corresponding allergen without acquiring clinical symptoms of food allergy (Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). Information on adverse reactions upon ingestion of endo-1,4-β-xylanase in individuals sensitised through the respiratory route has not been reported.

According to the information provided, substances or products that may cause allergies or intolerances (Regulation (EU) No 1169/2011[30]) are used as raw materials ([redacted])[31] in the media fed to the microorganisms. In addition, [redacted], which are known allergens, are also present in the media fed to the microorganisms. However, during the fermentation process, these products will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the microbial biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that potentially allergenic residues of these foods employed as protein sources are not expected to be present.

The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme cannot be excluded but the likelihood of such reactions to occur is considered to be low.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in two food processes.[32] Intended uses and the recommended use levels are summarised in Table 2.

[29] Technical dossier/1st submission/Section 3.2.2.2 and Annex U.

[30] 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.

[31] Additional information November 2019/Annex Z.

[32] Technical dossier/p. 64 and Additional information November 2019.

Table 2: Intended uses and recommended use levels of the food enzyme as provided by the applicant

Food manufacturing process[(a)]	Raw material	Recommended dosage of the food enzyme
Baking processes	Flour	Up to 8.28 mg TOS/kg flour
Grain treatment for the production of starch and gluten fractions	Grains	Up to 0.74 mg TOS/kg grains

TOS: total organic solids.
(a): The description has been harmonised according to the 'EC working document describing the food processes in which food enzymes are intended to be used' – not yet published at the time of adoption of this opinion.

In baking processes, the endo-1,4-β-xylanase is added to flour during the preparation of dough or batter handling. It hydrolyses (arabino)xylans, which interact with gluten and bind water, thus contributing to the reduction of dough viscosity. The decrease in viscosity facilitates the handling of the dough, gives improved crumb structure and increases the volume.

The food enzyme remains in the dough. Based on data provided on thermostability (see Section 3.3.1), it is anticipated that the endo-1,4-β-xylanase is inactivated during baking processes.

In grain treatment for the production of starch and gluten fractions, the endo-1,4-β-xylanase is added to grain during the initial steps, such as conditioning, homogenisation and dough preparation. It is used to reduce technical difficulties (e.g. high viscosity), to give higher yield due to efficient hydrolysis of xylans, improving starch purity due to greater extraction yield of the high-value fraction and efficient removal of fibres and proteins, resulting in more efficient processes and more consistent product quality.

3.5.2. Dietary exposure estimation

The technical information and experimental data provided on the removal of food enzyme–TOS during grain treatment for the fractionation of starch and gluten were considered by the Panel as sufficient to exclude this process from the exposure assessment (Annex B in EFSA CEF Panel, 2016). Consequently, a dietary exposure was not calculated for this process.

The dietary exposure to the food enzyme–TOS is calculated for the baking processes. Chronic exposure was calculated by combining the maximum recommended use level provided by the applicant (see Table 2) with the relevant FoodEx categories (Annex B in EFSA CEF Panel, 2016), based on individual consumption data. Exposure from individual FoodEx categories was subsequently summed up, averaged over the total survey period 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 one 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 the 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 35 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B).

Table 3: Summary of estimated dietary exposure to food enzyme–TOS in six population groups

Population group	Estimated exposure (mg TOS/kg body weight per day)					
	Infants	Toddlers	Children	Adolescents	Adults	The elderly
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0.002–0.023 (10)	0.017–0.050 (14)	0.020–0.048 (19)	0.011–0.030 (18)	0.008–0.019 (19)	0.008–0.017 (18)
Min–max 95th percentile (number of surveys)	0.009–0.099 (8)	0.044–0.084 (12)	0.039–0.090 (19)	0.024–0.062 (17)	0.018–0.037 (19)	0.016–0.029 (18)

TOS: total organic solids.

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	
FoodEx categories included in the exposure assessment were assumed to always contain the food enzyme–TOS	+
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 other processes from the exposure assessment – grain treatment for the production of starch and gluten fractions	–

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

The conservative approach applied to the exposure estimate to food enzyme–TOS, in particular assumptions made on the occurrence and use levels of this specific food enzyme, is likely to have led to a considerable overestimation of the exposure.

The exclusion of one food manufacturing process (grain treatment for the production of starch and gluten fractions – see Table 4) from the exposure assessment was based on > 99% of TOS removal during this process and is not expected to have an impact on the overall estimate derived.

3.6. Margin of exposure

Due to the absence of suitable toxicological data, the margin of exposure (MoE) could not be calculated.

4. Conclusions

As the production strain was not shown to meet the criteria for the QPS approach to safety assessment, and in the absence of suitable toxicological studies, the Panel cannot conclude on the safety of the food enzyme endo-1,4-β-xylanase from *B. subtilis* DP-Ezd31.

The production strain of the food enzyme contains multiple copies of an antimicrobial resistance gene. However, based on the absence of viable cells and DNA from the production organism in the food enzyme, this is not considered to be a risk.

5. Documentation as provided to EFSA

1) Application for authorisation of endo-1,4-beta-xylanase from a genetically modified strain of *Bacillus subtilis* DP-Ezd31'. March 2015. Submitted by Danisco US Inc.
2) Additional information. November 2019. Submitted by Danisco US Inc.
3) Summary report on GMM part for endo-1,4-beta-xylanase produced by *Bacillus subtilis* strain DP-Ezd31, EFSA-Q-2015-00839. Delivered by DTU (Denmark).
4) Additional information. December 2020. Submitted by Danisco US Inc.

References

Armentia A, Dıaz-Perales A, Castrodeza J, Duenas-Laita A, Palacin A and Fernandez S, 2009. Why can patients with baker's asthma tolerate wheat flour ingestion? Is wheat pollen allergy relevant? Allergologia et Immunopathologia, 37, 203–204. https://doi.org/10.1016/j.aller.2009.05.001

[redacted]

Brisman J, 2002. Baker's asthma. Occupational and Environmental Medicine, 59, 498–502, quiz 502, 426.

[redacted]

EFSA (European Food Safety Authority), 2006. Opinion of the Scientific Committee related to uncertainties in dietary exposure assessment. EFSA Journal 2006;5(1):438, 54 pp. https://doi.org/10.2903/j.efsa.2007.438

EFSA (European Food Safety Authority), 2009a. Guidance of EFSA prepared by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. EFSA Journal 2009;7(5):1305, 26 pp. https://doi.org/10.2903/j.efsa.2009.1305

EFSA (European Food Safety Authority), 2009b. Guidance of the Scientific Committee on Transparency in the Scientific Aspects of Risk Assessments carried out by EFSA. Part 2: general Principles. EFSA Journal 2009;7 (5):1051, 22 pp. https://doi.org/10.2903/j.efsa.2009.1051

EFSA (European Food Safety Authority), 2011. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011;9(3):2097, 34 pp. https://doi.org/10.2903/j.efsa.2011.2097

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2016. Panel statement on the exposure assessment of food enzymes. EFSA Journal 2016;14(11):4581, 9 pp. https://doi.org/10.2903/j.efsa.2016.4581. Available online: https://efsa.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.2903%2Fj.efsa.2016.4581&file=efs24581-sup-0001-Annex_B.pdf

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), 2019. Statement on the characterisation of microorganisms used for the production of food enzymes. EFSA Journal 2019;17(6):5741, 13 pp. https://doi.org/10.2903/j.efsa.2019.5741

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010. Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal 2010;8(7):1700, 168 pp. https://doi.org/10.2903/j.efsa.2010.1700

Elms J, Fishwick D, Walker J, Rawbone R, Jeffrey P, Griffin P, Gibson M and Curran AD, 2003. Prevalence of sensitisation to cellulase and xylanase in bakery workers. Occupational and Environmental Medicine, 60, 802–804. https://doi.org/10.1136/oem.60.10.802

FAO/WHO (Food and Agriculture Organization of the United States/World Health Organization), 2006. General specifications and considerations for enzyme preparations used in food processing in Compendium of food additive specifications. 67th meeting. FAO JECFA Monographs 3, pp. 63–67. Available online: ftp://ftp.fao.org/docrep/fao/009/a0675e/a0675e00.pdf

[redacted]

Martel C, Nielsen GD, Mari A, Licht TR and Poulsen LK, 2010. Bibliographic review on the potential of microorganisms, microbial products and enzymes to induce respiratory sensitization. EFSA Supporting Publication 2010;7(9):EN-75, 95 pp. https://doi.org/10.2903/sp.efsa.2010.en-75

Merget R, Sander I, Raulf-Heimsoth M and Baur X, 2001. Baker's asthma due to xylanase and cellulase without sensitization to alpha-amylase and only weak sensitization to flour. International Archives of Allergy and Immunology, 124, 502–505.

Poulsen LK, 2004. Allergy assessment of foods or ingredients derived from biotechnology, gene-modified organisms, or novel foods. Molecular Nutrition and Food Research, 48, 413–423. https://doi.org/10.1002/mnfr.200400029

[redacted]

Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
CFU	colony forming units
DRF	dose-range finding
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practice
GMM	genetically modified microorganism
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
MIC	Minimum Inhibitory Concentration
MoE	margin of exposure
NTG	*N*-Nitro-*N*-nitrosoguanidine
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
QPS	Qualified Presumption of Safety
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TOS	total organic solids
TSA	tryptic soy agar
WGS	whole genome sequence
WHO	World Health Organization

Appendix A – Dietary exposure estimates to the food enzyme–TOS in details

Information provided in this appendix is shown in an excel file (downloadable https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2021.6562).

The file contains two sheets, corresponding to two tables.

Table 1. Mean 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 one day
Infants	From 12 weeks on up to and including 11 months of age	Bulgaria, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, United Kingdom
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Spain, United Kingdom
Children[(a)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Portugal, Spain, Sweden, United Kingdom
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Spain, Sweden, United Kingdom
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Spain, Sweden, United Kingdom
The elderly[(a)]	From 65 years of age and older	Austria, Belgium, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Spain, Sweden, United Kingdom

(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).