SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 5 May 2021

doi: 10.2903/j.efsa.2021.6634

Safety evaluation of the food enzyme maltogenic α-amylase from the genetically modified *Bacillus subtilis* strain ROM

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Abstract

The food enzyme maltogenic α-amylase (glucan 1,4-α-maltohydrolase; EC 3.2.1.133) is produced with the genetically modified *Bacillus subtilis* strain ROM by DSM Food Specialities B.V. The genetic modifications do not give rise to safety concerns. The maltogenic α-amylase is considered free from viable cells of the production organism and its recombinant DNA. The food enzyme is intended to be used in baking processes. Based on the maximum use levels recommended for the baking processes and individual data from the EFSA Comprehensive European Food Database, dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.065 mg TOS/kg body weight (bw) per day. As the production strain of *B. subtilis* ROM qualifies for the Qualified Presumption of Safety approach to safety assessment and no issue of concern arose from the production process, no toxicological data are required. Similarity of the amino acid sequence of the food enzyme to those of known allergens was searched and six matches were 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. 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: food enzyme, glucan 1,4-α-maltohydrolase, EC 3.2.133, maltogenic α-amylase, *Bacillus subtilis*, genetically modified microorganism

Requestor: European Commission

Question number: EFSA-Q-2020-00583

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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.

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.

Acknowledgments: The Panel wishes to thank Simone Lunardi, Ivana Nikodinoska and Irene Nuin for the support provided to this scientific output.

Suggested citation: EFSA CCEP 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, Glandorf B, Herman L, Liu Y and Chesson A, 2021. Scientific Opinion on the safety evaluation of the food enzyme maltogenic α-amylase from the genetically modified *Bacillus subtilis* strain ROM. EFSA Journal 2021;19(6):6634, 14 pp. https://doi.org/10.2903/j.efsa.2021.6634

ISSN: 1831-4732

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

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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 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.

An application has been introduced by the applicant "DSM Food Specialities B.V." for the authorization of the food enzyme glucan 1,4-α-maltohydrolase produced from a genetically modified strain of *Bacillus subtilis* (strain ROM). The amino acid sequence of the protein has been modified, resulting in higher activity from glucan 1,4-α-maltohydrolase under the conditions of use.

Following the requirements of Article 12.1 of Commission Regulation (EU) 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

The European Commission requests the European Food Safety Authority to carry out the safety assessment on the following food enzyme: glucan 1,4-α-maltohydrolase produced from a genetically

[1] Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on Food Enzymes and Amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L 354, 31.12.2008, pp. 7–15.

[2] Regulation (EC) No. 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, pp. 1–6.

[3] Commission Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 64, 11.3.2011, pp. 15–24.

modified strain of *Bacillus subtilis* (strain ROM) in accordance with Article 29 of Regulation (EC) No 178/2002, and Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme glucan 1,4-α-maltohydrolase from a genetically modified *B. subtilis* strain ROM.

Additional information was requested from the applicant during the assessment process on 7 December 2020 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 guidance's 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 to the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016).

3. Assessment

IUBMB nomenclature:	Glucan 1,4-α-maltohydrolase
Systematic name:	4-α-D-glucan α-maltohydrolase
Synonyms:	Maltogenic α-amylase
IUBMB no.:	EC 3.2.1.133
CAS no.:	160611-47-2
EINECS no.:	Not available

The maltogenic α-amylase catalyses the hydrolysis of (1,4)-α-D-glucosidic linkages in starch polysaccharides, to successively remove maltose residues from the non-reducing ends of the chains.[4] The enzyme is intended to be used in baking processes.[5]

3.1. Source of the food enzyme

The maltogenic α-amylase is produced with the genetically modified *B. subtilis* strain ROM, which is deposited [redacted]

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 and toxigenic activity are verified for the specific strain used (EFSA, 2007; EFSA BIOHAZ Panel, 2020). [redacted]

[4] Technical dossier/1st submission/pp. 36.
[5] Technical dossier/1st submission/pp. 51–53.
[6] Technical dossier/1st submission/Annex II-10.
[7] Technical dossier/1st submission/Annex II-2.
[8] Technical dossier/1st submission/Annex II-5.

[redacted]

3.1.1. Characteristics of the parental and recipient microorganisms

The parental microorganism is [redacted]

3.1.2. Characteristics of introduced sequences

The sequence encoding the maltogenic α-amylase [redacted] is a variant of the wild-type gene [redacted] The encoded protein contains four amino acid substitutions to improve its enzymatic activity. [redacted]

3.1.3. Description of the genetic modification process

[redacted]

[9] Technical dossier/1st submission/Annexes II-6, II-7 and II-8.

[redacted]

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* ROM differs from the recipient strain [redacted] in its capacity to produce the maltogenic α-amylase [redacted]

The absence of the antibiotic resistance genes used during the genetic modifications was confirmed [redacted]

Since the introduced genetic modifications do not raise safety concerns and no cytotoxic activity is present, the QPS approach can be applied to the production strain (EFSA BIOHAZ Panel, 2020).

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[12], with food safety procedures based on hazard analysis and critical control points, and in accordance with current Good Manufacturing Practice.[13]

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, cells are killed and 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 weight material passes the filtration membrane and is discarded.[14] 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.[15]

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 maltogenic α-amylase is a single polypeptide chain of 686 amino acids. The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be 75 kDa.[16] The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE).[17] A consistent protein pattern was observed across all batches. The gels showed a single major protein band corresponding to an apparent molecular mass of about 66 kDa, consistent with the expected mass of the enzyme. The protein profile also included bands of lower staining intensity. No other enzyme activities were reported.[18]

The in-house determination of activity is based on hydrolysis of the substrate maltotriose (reaction conditions: pH 5.0, 40°C, 10 min), spectrophotometrically measuring the release of glucose by a

[10] Technical dossier/1st submission/Annex II-9.
[11] Technical dossier/1st submission/Annex II-2 and Annex II-3.
[12] 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.
[13] Technical dossier/1st submission/Annex I-5.
[14] Technical dossier/1st submission/pp. 41-47 and Annex I-6.
[15] Technical dossier/1st submission/Annex I-7.
[16] Technical dossier/1st submission/pp. 35 and 78.
[17] Technical dossier/1st submission/pp. 33.
[18] Technical dossier/1st submission/pp. 33-34.

hexokinase assay. The enzyme activity is expressed in RMAU/g. One unit of maltogenic α-amylase activity (RMAU) is defined as the amount of enzyme required to release 0.5 mg glucose from maltotriose under the assay conditions.[19]

The food enzyme has a temperature optimum around 60°C (pH 5) and a pH optimum around pH 5 (37°C). At pH 5, the enzyme activity decreased above 90°C, showing no residual activity above 96.6°C after 15 min incubation.[20]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (Table 1).[21] The average total organic solids (TOS) of the three food enzyme batches was 11.0% and the average enzyme activity/TOS ratio 66.0 RMAU/mg TOS.

Table 1: Compositional data of the food enzyme

Parameters	Unit	Batches		
		1	2	3
Maltogenic amylase activity	RMAU/g batch[(a)]	6,820	6,310	8,325
Protein	%	6.64	4.99	7.36
Ash	%	1.34	1.24	2.36
Water	%	87.41	90.22	84.56
Total organic solids (TOS)[(b)]	%	11.25	8.54	13.08
Activity/mg TOS	RMAU/mg TOS	60.6	73.9	63.6

(a): RMAU: Maltogenic amylase units (see Section 3.3.1).
(b): TOS calculated as 100% – % water – % ash.

3.3.3. Purity

The lead content[21] in the three batches was up to 0.01 mg/kg, 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, *Escherichia 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 these batches (FAO/WHO, 2006).[21]

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 viable cells of the production strain in the food enzyme at the end of the killing was demonstrated [redacted] No colonies were produced.[22]

The absence of recombinant DNA in the food enzyme was demonstrated [redacted] No DNA was detected [redacted]

3.4. Toxicological data

As the production strain qualifies for the QPS approach of safety assessment and no issue of concern arising from the production process of the food enzyme were identified (see Sections 3.1, 3.2 and 3.3), the Panel considers that no toxicological studies other than assessment of allergenicity are necessary.[24]

[19] Technical dossier/1st submission/p.36 and Annex I-2.
[20] Technical dossier/Additional data February 2021.
[21] Technical dossier/Additional data February 2021/Annex 1.
[22] Technical dossier/1st submission/pp. 88–89.
[23] Technical dossier/1st submission/Annex II-12.
[24] Article 1.2 of the Regulation (EU) No 562/2012 of 27 June 2012 amending Commission Regulation (EU) No 234/2011.

3.4.1. Allergenicity

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

The potential allergenicity of the maltogenic α-amylase produced with the genetically modified *B. subtilis* strain ROM 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, six matches were found. The matching allergens were: Asp o 21 (TAKA amylase A from *Aspergillus oryzae*) and Asp o 21.0101 (alpha-amylase A type-1/2 from *Aspergillus oryzae*), Sch c 1.0101 (glycoside hydrolase family 15 from *Schizophyllum commune* or Split Gill fungus), Aed a 4.0101 (probable maltase from *Aedes aegypti* or yellow fever mosquito), Asp f 13.0101 (uncleaved alkaline protease from *Aspergillus fumigatus*) and Asp f 13 (partial alkaline protease from *Aspergillus fumigatus*).[25]

No information is available on oral and respiratory sensitisation or elicitation reactions of this maltogenic α-amylase.

The α-amylase from *A. oryzae* (Brisman and Belin, 1991; Quirce et al., 1992, 2002; Sander et al., 1998; Brisman, 2002), serine protease from *A. fumigatus* (Kurup et al., 2002) and glucoamylase from *S. commune* (Toyotome et al., 2014) are known as occupational respiratory allergens associated with asthma. However, several studies have shown that adults with occupational asthma to a food enzyme (as described for α-amylase from *A. oryzae*) can ingest respiratory allergens without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Poulsen, 2004; Armentia et al., 2009). Taking into account the wide use of α-amylase as a food enzyme, only a low number of case reports has been described in the literature that focused on allergic reactions upon oral exposure to α-amylase in individuals respiratory-sensitised to α-amylase (Losada et al., 1992; Quirce et al., 1992; Baur and Czuppon, 1995; Kanny and Moneret-Vautrin, 1995; Moreno-Ancillo et al., 2004). Such information has not been reported for glucoamylase and serine protease. The serine protease produced by *S. commune* is associated with allergic reactions to mites and insect bites, while maltase from the yellow fever mosquito is also associated with bites, but no effects of oral exposure to this enzyme have been reported. In addition, no allergic reactions upon dietary exposure to any maltogenic α-amylase have been reported in the literature.

[redacted], a known allergen, was used as a raw material in the media fed to the microorganisms.[15] 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 of this material [redacted] are not expected to be present.

The Panel concluded that an allergic reaction upon oral ingestion of maltogenic α-amylase, produced by the genetically modified *B. subtilis* strain ROM, in individuals respiratory-sensitised to α-amylase, serine protease produced by *A. fumigatus* or glucoamylase produced by *S. commune* cannot be excluded, but the likelihood 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 baking processes at the maximum use level of up to 5.5 mg TOS/kg flour.[26]

In baking processes, the food enzyme performs its technological function during dough or batter handling, contributing to an improved and consistent baking process.[27] The conversion of starch lowers the rate of retrogradation, thereby reducing staling, and improves crumb structure.[28] Based on data provided on thermostability (see Section 3.3.1), it is expected that the maltogenic α-amylase is inactivated during the baking step.

[25] Technical dossier/1st submission/pp. 57 and Annex I-8.
[26] Technical dossier/1st submission/pp. 53–54.
[27] Technical dossier/1st submission/pp. 64–65.
[28] Technical dossier/1st submission/pp. 66–67.

3.5.2. Dietary exposure estimation

Chronic exposure was calculated by combining the maximum recommended use level provided by the applicant 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 average 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 2 provides an overview of the derived exposure estimates across all surveys. Detailed average 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 35 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). The highest dietary exposure to the food enzyme-TOS was estimated to be up to 0.065 mg TOS/kg body weight (bw) per day in infants.

Table 2: 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)	0.001–0.015 (10)	0.012–0.033 (14)	0.013–0.032 (19)	0.007–0.020 (18)	0.005–0.013 (19)	0.005–0.011 (18)
Min–max 95th percentile (number of surveys)	0.006–0.065 (8)	0.029–0.056 (12)	0.026–0.060 (19)	0.016–0.041 (17)	0.012–0.025 (19)	0.011–0.020 (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 3.

Table 3: 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	+/–

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.

3.6. Margin of exposure

Since no toxicological assessment was considered necessary by the Panel, the margin of exposure was not calculated.

4. Conclusions

Based on the data provided, the Panel concluded that the food enzyme maltogenic α-amylase produced with the genetically modified *B. subtilis* strain ROM does not give rise to safety concerns under the intended conditions of use.

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

5. Documentation as provided to EFSA

1) "Application for authorization of glucan 1,4-α-maltohydrolase from a genetically modified strain of *Bacillus subtilis* in accordance with Regulation (EC) No 1331/2008", August 2020. Submitted by DSM Food Specialities.
2) "Additional information on glucan 1,4-α-maltohydrolase from the genetically modified *Bacillus subtilis* strain ROM EFSA-Q-2020-00583", 03 February 2021. Submitted by DSM Food Specialities.

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Abbreviations

ANI	Average Nucleotide Identity
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
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
MIC	minimum inhibitory concentration
PCR	polymerase chain reaction
QPS	Qualified Presumption of Safety
RMAU	Maltogenic amylase units
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	total organic solids
WGS	whole genome sequencing
WHO	World Health Organization

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

Information provided in this appendix is shown in an excel file (downloadable https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa..2021.6634).

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).