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Safety evaluation of the food enzyme β-galactosidase from the non-genetically modified *Kluyveromyces lactis* strain GD-YNL

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Abstract

The food enzyme β-galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23) is produced with the non-genetically modified *Kluyveromyces lactis* strain GD-YNL by Godo Shusei Co., Ltd. The food enzyme is intended to be used for the hydrolysis of lactose in milk processing, production of fermented milk products and whey processing. The food enzyme is also intended for lactose hydrolysis in milk products at home. Dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 54 mg TOS/kg body weight per day in European populations. As the production strain qualifies for the qualified presumption of safety approach of safety assessment and as no issue of concern raised from the production process, no toxicological studies other than assessment of allergenicity were necessary. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and no match was found. The Panel considered that, under the intended conditions of use, the risk of allergic reactions by dietary exposure cannot be excluded, but the likelihood is 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: β-Galactosidase, β-D-galactoside galactohydrolase, EC 3.2.1.23, lactase, *Kluyveromyces lactis*

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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 microorganisms or products thereof including a product obtained by a fermentation process using microorganisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.

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

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

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

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

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 by the companies "Danisco US Inc." for the authorisation of the food enzyme Hexose oxidase from a genetically modified strain of *Hansenula polymorpha* (strain DP-Jza21); "Novozymes A/S." for the authorisation of the food enzyme Pectin lyase from a genetically modified strain of *Aspergillus niger* (strain NZYM-PN); "Puratos NV" for the authorisation of the food enzyme Xylanase from a genetically modified strain of *Bacillus subtilis* (strain LMG-S-27588); the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) for the authorisation of the food enzyme Beta-galactosidase from *Kluyveromyces lactis* and "AB Enzymes GmbH" for the authorisation of the food enzyme Lysophospholipase from a genetically modified strain of *Trichoderma reesei* (strain RF7206).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008, the Commission has verified that the five applications fall within the scope of the food enzyme Regulation and contain 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, pp. 15–24.

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 Hexose oxidase from a genetically modified strain of *Hansenula polymorpha* (strain DP-Jza21), Pectin lyase from a genetically modified strain of *Aspergillus niger* (strain NZYM-PN), Xylanase from a genetically modified strain of *Bacillus subtilis* (strain LMG-S-27588), Beta-galactosidase from *Kluyveromyces lactis* and Lysophospholipase from a genetically modified strain of *Trichoderma reesei* (strain RF7206) 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 β-galactosidase from *Kluyveromyces lactis*. The application was submitted initially as a joint dossier[4] and identified as EFSA-Q-2015-00409. During an ad hoc meeting between EFSA, the European Commission and the Association of Manufacturers and Formulators of Enzyme Products (AMFEP),[5] it was agreed that joint dossiers will be split into individual data packages.

The current opinion addresses one data package originating from the joint dossier EFSA-Q-2015-00409. This data package, identified as EFSA-Q-2021-00469, concerns the food enzyme β-galactosidase that is produced with a strain of *Kluyveromyces lactis* and submitted by Godo Shusei Co., Ltd.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme β-galactosidase from *Kluyveromyces lactis* strain GD-YNL.

Additional information was requested from the applicant during the assessment process on 11 October 2021, 11 October 2021 and 4 July 2022, 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) and following the relevant existing guidance of EFSA Scientific Committees.

The current 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) as well as the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) have been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance to the methodology described in the CEF Panel 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021).

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

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

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

β-Galactosidases catalyse the hydrolysis of the β-(1,4)-glycosidic linkage of lactose (β-D-galactosyl 1,4-D-glucoside) resulting in the generation of D-galactose and D-glucose. The food enzyme is intended to be used for the lactose hydrolysis in milk processing, production of fermented milk products and whey processing. The food enzyme is also intended for lactose hydrolysis in milk products at home.

3.1. Source of the food enzyme

The β-galactosidase is produced with the yeast *Kluyveromyces lactis* strain GD-YNL, which is deposited at the National Institute of Technology and Evaluation (NITE) Biological Resource Center (Japan) with the deposit number NITE SD 00457.[6] The production strain was identified as *K. lactis* by sequence identity of the D1/D2 domain of the 26 S rRNA gene with the type strains of *K. lactis* var. *lactis* and *K. lactis* var. *drosophilarum*.[7] *Kluyveromyces lactis* GD-YNL is not genetically modified.

The species *K. lactis* is included in the list of organisms for which the qualified presumption of safety[8] (QPS; EFSA BIOHAZ Panel, 2020) may be applied. The production strain has been non-equivocally identified as *K. lactis* and therefore 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,[9] with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current good manufacturing practice.[10]

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 cells are physically disrupted to release the intracellular enzyme. The solid biomass is removed by filtration, leaving a filtrate containing the food enzyme. The filtrate is then further purified and concentrated, including an ultrafiltration step in which enzyme protein is retained, while most of the low molecular mass material passes the filtration membrane and is discarded.[11,12]

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 1,025 amino acids.[13] The molecular mass of the mature protein, calculated from the amino acid sequence, was 117.6 kDa.[14] The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). A consistent protein pattern was observed across all batches. The gel showed a single major protein band corresponding to an apparent molecular mass of about 120 kDa,[15] consistent with the expected mass of the enzyme. The food enzyme was tested for lipase, protease and invertase activities, and protease and invertase activities were detected.[16] No other enzymatic activities were reported.

The determination of β-galactosidase activity is based on hydrolysis of *o*-nitrophenyl-β-D-galactopyranoside (reaction conditions: pH 6.5, 30°C, 10 min). The enzymatic activity is determined by measuring the release of *o*-nitrophenol spectrophotometrically at 420 nm. The enzyme activity is expressed in Neutral Lactase Unit (NLU)/g. One NLU is defined as the quantity of enzyme that releases 1.30 μmol/min of *o*-nitrophenol under the conditions of the assay.[17]

[6] Technical dossier/Annex K.
[7] Technical dossier/Additional data February 2022/Annex L.
[8] https://zenodo.org/record/6902983#.Y4StlnbMKUm
[9] 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.
[10] Technical dossier/p. 34 and Annex E.
[11] Technical dossier/pp. 34–42 and Annex F.
[12] Technical dossier/Additional data February 2022/Annex G (revised).
[13] Technical dossier/p. 26.
[14] Technical dossier/Additional data February 2022/220218 b-galactosidase from *K.lactis*(GODO-YNL2) + Dossier/p. 28.
[15] Technical dossier/p. 24.
[16] Technical dossier/Annexes B and C.
[17] Technical dossier/p. 27 and Annex B.

The food enzyme has a temperature optimum around 45°C (pH 6.5) and a pH optimum around pH 6.5 (30°C). Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures. β-Galactosidase activity was stable up to 40°C. With the increasing temperature, activity was reduced, with no activity detected at pre-incubation temperatures over 50°C.[18]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme preparation were provided for three batches used for commercialisation (Table 1).[19] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation was 7.7% and the mean enzyme activity/TOS ratio was 70.4 NLU/mg TOS.

Table 1: Composition of the food enzyme preparation

Parameters	Unit	Batches		
		1	2	3
β-galactosidase activity	NLU/g batch[(a)]	5,450	5,300	5,500
Protein	%	3.7	3.7	3.7
Ash	%	2.6	2.6	2.6
Water	%	39.5	39.7	39.9
Glycerol (excipient)	%	50.0	50.0	50.0
Total organic solids (TOS)[(b)]	%	7.9	7.7	7.5
Activity/TOS	NLU/mg TOS	69.0	68.8	73.3

(a): NLU: Neutral Lactase Unit (see Section 3.3.1).
(b): TOS calculated as 100% – % water – % ash – % excipient.

3.3.3. Purity

The lead content in the three commercial batches was below 0.2 mg/kg,[20] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). In addition, the level of arsenic was below the limit of detection (LoD) of the employed method.[21,22]

The food enzyme preparation complies with the microbiological criteria (for total coliforms, *Escherichia coli* and *Salmonella*) as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[23] No antimicrobial activity was detected in any of the tested batches.[24]

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

3.4. Toxicological data

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

3.4.1. Allergenicity

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

The potential allergenicity of the β-galactosidase produced with the non-genetically modified *Kluyveromyces lactis* strain GD-YNL 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

[18] Technical dossier/ Additional data February 2022/220218 b-galactosidase from *K.lactis*(GODO-YNL2) + Dossier/p. 29.
[19] Technical dossier/p. 22, Annexes A, B, C and additional data February 2022/Annex M.
[20] Technical dossier/p. 25 and Annexes C and D.
[21] Technical dossier/Annexes C and D.
[22] LoDs: Pb = 0.2 mg/kg; As = 0.1 mg/kg.
[23] Technical dossier/p. 25/Annexes C and D.
[24] Technical dossier/Additional data February 2022/Annex N.

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.[25]

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

Cases of occupational allergy following exposure by inhalation of β-galactosidase have been reported (Stöcker et al., 2016). However, several studies have shown that adults with occupational asthma can ingest respiratory allergens without acquiring clinical symptoms of food allergy (Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). In addition, two case reports describing allergic reactions (swollen throat, shortness of breath and difficulty in swallowing) following ingestion of β-galactosidase pills, and confirmation by antigen challenge, have been reported (Binkley, 1996; Voisin and Borici-Mazi, 2016).

Corn steep liquor and yeast extract, known sources of allergens, are 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 fungal biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that no potentially allergenic residues are present in the food enzyme.

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

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in three food manufacturing processes and for home use 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

Food manufacturing process[(a)]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[(b)]
Lactose hydrolysis in milk processing	Milk	6–**590**
Production of fermented milk products	Milk	6–**590**
Whey processing	Liquid whey	6–**590**
Lactose hydrolysis in milk products at home	Infant formula, follow-on formula and other milk products	26.2–**66**

Technical dossier/pp.45–49 and Additional data September 2022/Summary of the response content (EFSA-Q-2021-00469).
(a): The name 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.
(b): The numbers in bold were used for calculation.

Two different dairy materials can be treated with this food enzyme: milk or whey. ß-Galactosidase hydrolyses lactose to release glucose and galactose. The treatment makes milk more suitable for lactose-intolerant individuals and sweeter.[26] Adding ß-galactosidase together with microbial cultures during fermentation would result in lactose-reduced yoghurt.[27] Treatment of the cheese whey or whey permeate would result in lactose-reduced and sweeter whey syrups.[28] No separation step is applied to remove the food enzyme–TOS from the treated milk, fermented milk products or whey syrup.

The enzymatically treated milk or whey can be consumed directly, but can also be used as ingredient in a large variety of foods. This includes infant formula and follow-on formula and foods for special medical purposes.[29] The enzymatic treatment also prevents the sandiness caused by lactose crystallisation in frozen desserts such as ice cream.

[25] Technical dossier/pp. 54–56 and Annex J.
[26] Technical dossier/Additional data February 2022/220218 b-galactosidase from *K.lactis*(GODO-YNL2) + Dossier/p.46.
[27] Technical dossier/Additional data February 2022/220218 b-galactosidase from *K.lactis*(GODO-YNL2) + Dossier/p.47.
[28] Technical dossier/Additional data February 2022/220218 b-galactosidase from *K.lactis*(GODO-YNL2) + Dossier/p.48.
[29] Technical dossier/Additional data February 2022/220218 b-galactosidase from *K.lactis*(GODO-YNL2) + Dossier/p.72.

The applicant provided a lower use level for treating milk at home.[29] Consumers may choose to add β-galactosidase to milk in order to produce lactose-reduced milk or yoghurt. The lower use level compared to the one applied in industrial applications is due to longer processing time at home when compared to industrial dairy processing.

Based on data provided on thermostability (see Section 3.3.1), it is expected that the ß-galactosidase is inactivated during the pasteurisation step.

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, 2021). 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 41 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). The highest dietary exposure was estimated to be about 54 mg TOS/kg bw per day in infants at the 95th percentile.

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)	1.155–14.583 (11)	1.649–21.803 (15)	4.407–18.757 (19)	0.844–6.946 (21)	0.882–3.041 (22)	0.339–2.739 (22)
Min–max 95th percentile (number of surveys)	5.042–53.898 (9)	22.931–50.471 (13)	10.108–31.073 (19)	2.961–14.567 (20)	2.670–8.788 (22)	2.712–6.306 (21)

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	+/–
Consumption survey for infants below 3 months of age are not included, due to limited availability	+/–

Sources of uncertainties	Direction of impact
Model assumptions and factors	
The use of an assumption that the 50% of dairy protein in regular infant formula and follow-on formula are from milk and 50% from whey	+/–
Exposure from whey processing considered both cheese whey and acid whey as raw material, although this food enzyme targets only cheese whey	+
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 to disaggregate FoodEx categories	+/–
Use of technical factors in the exposure model	+/–

+: 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 overestimation of the exposure.

3.6. Margin of exposure

Since toxicological assessment was considered unnecessary by the Panel, the margin of exposure was not calculated.

4. Conclusions

Based on the data provided, the QPS status of the production strain and the absence of issues of concern arising from the production process, the Panel concluded that the food enzyme β-galactosidase produced with *Kluyveromyces lactis* strain GD-YNL does not give rise to safety concerns under the intended conditions of use.

5. Documentation as provided to EFSA (if appropriate)

Application for authorisation of *β-galactosidase* from *Kluyveromyces lactis* in accordance with Regulation (EC) No 1331/2008. August 2021. Submitted by Godo Shusei Co., Ltd.

Additional information January 2022. Submitted by Godo Shusei Co., Ltd.

Additional information February 2022. Submitted by Godo Shusei Co., Ltd.

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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
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organisation of the United Nations
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
QPS	Qualified presumption of safety
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TOS	total organic solids
WHO	World Health Organisation

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.2023.7750#support-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
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, 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, Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, 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, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden

(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).