SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 6 June 2018

doi: 10.2903/j.efsa.2018.5319

Safety evaluation of the food enzyme glucose oxidase from a genetically modified *Aspergillus oryzae* (strain NZYM-KP)

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), Vittorio Silano, Claudia Bolognesi, Laurence Castle, Kevin Chipman, Jean-Pierre Cravedi, Paul Fowler, Roland Franz, Konrad Grob, Rainer Gürtler, Trine Husøy, Sirpa Kärenlampi, Wim Mennes, Maria Rosaria Milana, Karla Pfaff, Gilles Riviere, Jannavi Srinivasan, Maria de Fátima Tavares Poças, Christina Tlustos, Detlef Wölfle, Holger Zorn, Andrew Chesson, Boet Glandorf, Lieve Herman, Klaus-Dieter Jany, Francesca Marcon, André Penninks, Andrew Smith, Henk van Loveren, Davor Želježic, Magdalena Andryszkiewicz, Yi Liu, Annamaria Rossi and Karl-Heinz Engel

Abstract

The food enzyme is a glucose oxidase (beta-D-glucose:oxygen 1-oxidoreductase; EC 1.1.3.4) produced with a genetically modified strain of *Aspergillus oryzae* strain NZYM-KP by Novozymes A/S. The genetic modifications do not give rise to safety concerns. The food enzyme does not contain the production organism or DNA; therefore, there is no safety concern for the environment. The glucose oxidase is intended to be used in baking processes. Based on the maximum use levels recommended and individual consumption data from the EFSA Comprehensive European Food Consumption Database, dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.156 mg TOS/kg body weight (bw) per day in European populations. The food enzyme did not induce gene mutations in bacteria or chromosome aberrations in human lymphocytes. The subchronic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rodents. A no-observed-adverse-effect level was derived (341 mg TOS/kg bw per day), which compared with the estimated dietary exposure results in a sufficiently high margin of exposure. The allergenicity was evaluated by comparing the amino acid sequence to those of known allergens and one match with a fungal contact allergen was found. The Panel considered that, under the intended condition of use, the risk of allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but the likelihood is considered low. Based on the microbial source, the genetic modifications, the manufacturing process, the compositional and biochemical data, the estimated dietary exposure and the findings in the toxicological studies, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

Keywords: food enzyme, glucose oxidase, beta-D-glucose:oxygen 1-oxidoreductase, EC 1.1.3.4, *Aspergillus oryzae*, genetically modified microorganism

Requestor: European Commission

Question number: EFSA-Q-2013-00687

Correspondence: fip@efsa.europa.eu

Panel members: Claudia Bolognesi, Laurence Castle, Kevin Chipman, Jean-Pierre Cravedi, Karl-Heinz Engel, Paul Fowler, Roland Franz, Konrad Grob, Rainer Gürtler, Trine Husøy, Sirpa Kärenlampi, Wim Mennes, Maria Rosaria Milana, Karla Pfaff, Gilles Riviere, Vittorio Silano, Jannavi Srinivasan, Maria de Fátima Tavares Poças, Christina Tlustos, Detlef Wölfle 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.

Acknowledgements: The Panel wishes to thank EFSA staff member(s): Margarita Aguilera-Gómez, Natália Kovalkovicová and Kim Rygaard Nielsen (deceased) for the support provided to this scientific output.

Suggested citation: EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (EFSA CEF Panel), Silano V, Bolognesi C, Castle L, Chipman K, Cravedi J-P, Fowler P, Franz R, Grob K, Gürtler R, Husøy T, Kärenlampi S, Mennes W, Milana MR, Pfaff K, Riviere G, Srinivasan J, Tavares Poças MF, Tlustos C, Wölfle D, Zorn H, Chesson A, Glandorf B, Herman L, Jany K-D, Marcon F, Penninks A, Smith A, van Loveren H, Želježic D, Andryszkiewicz M, Liu Y, Rossi A and Engel K-H, 2018. Scientific Opinion on the safety evaluation of the food enzyme glucose oxidase from a genetically modified *Aspergillus oryzae* (strain NZYM-KP). EFSA Journal 2018;16(7):5319, 20 pp. https://doi.org/10.2903/j.efsa.2018.5319

ISSN: 1831-4732

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

Table of contents

Abstract 1
1. Introduction 4
1.1. Background and Terms of Reference as provided by the requestor 4
1.1.1. Background as provided by the European Commission 4
1.1.2. Terms of Reference 4
1.2. Interpretation of the Terms of Reference 5
1.3. Information on existing authorisations and evaluations 5
2. Data and methodologies 5
2.1. Data 5
2.2. Methodologies 5
3. Assessment 5
3.1. Technical data 5
3.1.1. Identity of the food enzyme 5
3.1.2. Chemical parameters 5
3.1.3. Properties of the food enzyme 6
3.1.4. Information on the source material 7
3.1.4.1. Information related to the genetically modified microorganism 7
3.1.4.2. Characteristics of the parental and recipient microorganisms 7
3.1.4.3. Characteristics of introduced sequences 7
3.1.4.4. Description of the genetic modification process 7
3.1.4.5. Safety aspects of the production strain 8
3.1.5. Manufacturing process 8
3.1.6. Safety for the environment 8
3.1.7. Case of need and intended conditions of use 8
3.1.8. Reaction and fate in food 9
3.2. Dietary exposure 9
3.2.1. EFSA Comprehensive European Food Consumption Database 9
3.2.2. Exposure assessment methodology 9
3.2.3. Exposure to food enzyme–TOS according to the intended use proposed by the applicant 10
3.2.4. Uncertainty analysis 10
3.3. Toxicological data 11
3.3.1. Genotoxicity 11
3.3.1.1. Bacterial reverse mutation test 11
3.3.1.2. In vitro mammalian chromosome aberration test 12
3.3.2. Repeated dose 90-day oral toxicity study in rodents 12
3.4. Allergenicity 13
Conclusions 13
Documentation provided to EFSA 14
References 14
Abbreviations 15
Appendix A – Population groups considered for the exposure assessment 16
Appendix B – FoodEx categories used to derive exposure estimates for the food enzyme–TOS and the respective conversion factors 17
Appendix C – Dietary exposure estimates to the food enzyme–TOS in details 20

1. Introduction

Article 3 of the Regulation (EC) No 1332/2008[1] provides definitions 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 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:

i) it does not pose a safety concern to the health of the consumer at the level of use proposed,
ii) there is a reasonable technological need, and
iii) its use does not mislead the consumer.

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

The 'Guidance on submission of a dossier on food enzymes for safety evaluation' (EFSA CEF Panel, 2009) 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 Union list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7 (2) of Regulation (EC) No 1332/2008 on food enzymes.

Three applications have been introduced by the company Novozymes A/S for the authorisation of the food enzymes Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-BC), Amyloglucosidase from a genetically modified strain of *Aspergillus niger* (strain NZYM-BR) and Glucose oxidase from a genetically modified strain of *Aspergillus oryzae* (strain NZYM-KP).

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 assessments on the food enzymes Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-BC), Amyloglucosidase from a genetically modified strain of *Aspergillus niger*

[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, p. 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, p. 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, p. 15–24.

(strain NZYM-BR) and Glucose oxidase from a genetically modified strain of *Aspergillus oryzae* (strain NZYM-KP) 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 the food enzyme glucose oxidase from a genetically modified strain of *Aspergillus oryzae* (strain NZYM-KP).

1.3. Information on existing authorisations and evaluations

The applicant reports that Danish and French authorities have evaluated and authorised the use of glucose oxidase from a genetically modified strain of *A. oryzae* (strain NZYM-KP) in baking processes. The Danish authority also sets out the conditions of use, including the dosages for specific foods, which is up to 500 GODU/kg flour.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier supporting the application for authorisation of the food enzyme glucose oxidase from a genetically modified strain of *A. oryzae* (strain NZYM-KP). The food enzyme is intended to be used in baking processes.

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA 'Guidance on transparency in the scientific aspects of risk assessment' (EFSA, 2009) and following the relevant existing guidances from the EFSA Scientific Committee.

The current 'Guidance on submission of a dossier on food enzymes for safety evaluation' (EFSA CEF Panel, 2009) 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

3.1. Technical data

3.1.1. Identity of the food enzyme

IUBMB nomenclature:	Glucose oxidase
Systematic name:	Beta-D-glucose:oxygen 1-oxidoreductase
Synonyms:	Beta-D-glucose oxidase; beta-D-glucose:quinone oxidoreductase
IUBMB No:	EC 1.1.3.4
CAS No:	9001-37-0
EINECS No:	232-601-0

3.1.2. Chemical parameters

The glucose oxidase produced with the genetically modified *A. oryzae* strain NZYM-SP is a single polypeptide chain of 581 amino acids. The molecular mass derived from the amino acid sequence was calculated to be 63 kDa. The protein homogeneity status of the food enzyme was investigated by sodium dodecyl sulfate-poly acrylamide gel electrophoresis (SDS-PAGE) analysis. The apparent molecular mass based on this technique is about 82–88 kDa identified with one band. The difference is expected to be due to glycosylation.

The food enzyme has been tested for other enzymatic activities. The protease, alpha-amylase, lipase and glucoamylase activities were below the detection limits of the employed methods. The food enzyme shows catalase activity, according to the applicant, the average activity of catalase determined in the three commercial batches is substantially lower than the activities in commercial catalase food enzymes.

Data on the chemical parameters of the food enzyme have been provided for three commercial batches and one batch used for the toxicological tests (Table 1). The average total organic solids (TOS) content of the three commercial food enzyme batches was 9.8% (w/w); the values ranged from 8.9% to 11.4% (Table 1). The TOS content is a calculated value derived as 100% minus % water minus % ash.

The enzyme activity/TOS ratio of the three commercial food enzyme batches ranged from 50.53 to 93.71 GODU/mg TOS (Table 1). The average value of 72 GODU/mg TOS was used for subsequent calculations.

Table 1: Compositional data provided for the food enzyme

Parameter	Unit	Batch			
		1	2	3	4[(a)]
Glucose oxidase activity	GODU/g batch[(b)]	8,340	6,450	5,760	4,790
Protein	%	7.44	6.50	7.13	4.88
Ash	%	2.5	2.4	2.3	2.4
Water	%	88.6	88.6	86.3	86.9
Total organic solids (TOS)[(c)]	%	8.9	9.0	11.4	10.7
Glucose oxidase activity/mg TOS	GODU/mg TOS	93.7	71.7	50.5	44.7

(a): Batch used for the toxicological tests.
(b): GODU: Glucose Oxidase Units (see Section 3.1.3).
(c): TOS calculated as 100% – % water – % ash.

The food enzyme complies with the specification for lead (not more than 5 mg/kg) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006). In addition, the levels of arsenic, cadmium and mercury in the four batches were below the limits of detection of the employed methodologies.[4]

The presence of secondary metabolites (beta-nitropropionic acid and cyclopiazonic acid) was examined in the four food enzyme batches and they were below the limits of detection of the applied analytical methods.

The food enzyme complies with the microbiological criteria as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006), which stipulate that *Escherichia coli* and *Salmonella* species are absent in 25 g of sample and total coliforms should not exceed 30 colony forming units (CFU) per gram.

The applicant has provided information on the identities of the antifoam agents used. Taking into account the nature and properties of the antifoam agents, the manufacturing process and the quality assurance system implemented by the applicant, the Panel considers their use as of no safety concern.

The Panel considered the compositional data provided for the food enzyme as sufficient.

3.1.3. Properties of the food enzyme

The glucose oxidase catalyses the oxidation of β-D-glucose to D-glucono-δ-lactone and hydrogen peroxide using molecular oxygen as acceptor of electrons.

The glucose oxidase activity is expressed in Glucose Oxidase Units/g (GODU/g) (reaction conditions: pH 5.6, temperature 30°C, reaction time 30 min). One unit of GODU is defined as the amount of enzyme which oxidises 1 μmol of β-D-glucose in 1 min. In the assay, the reaction catalysed by the glucose oxidase is coupled with a second one catalysed by a peroxidase. This peroxidase uses the formed hydrogen peroxide to oxidise 2,2-azino-di-(3-ethylbenzthiazoline)-6-sulfonate (ABTS). The oxidation of the latter substrate is measured spectrophotometrically at 405 nm and is proportional to the amount of glucose oxidised during the first reaction.

Data showed that catalase activity was not sufficient to interfere with the determination of food enzyme activity.

The glucose oxidase has been characterised regarding its activity depending on temperature and pH. The glucose oxidase is active at temperatures up to 70°C (with an optimum of 50–60°C at pH 6) and within a pH range of 2–10 (with an optimum of pH 3–5.5 at 30°C). The thermostability of the food enzyme was tested over a range of 25–90°C after a pre-incubation at the different temperatures for

[4] Limit of detection: Pb: 0.5 mg/kg; As: 0.1 mg/kg; Cd: 0.05 mg/k; Hg: 0.03 mg/kg.

30 min at pH 6. The activity itself was measured at pH 6 and 30°C. The food enzyme retained its activity at temperatures up to 60°C. At higher temperatures, the food enzyme rapidly loses its activity; above 80°C, no activity remains after 30 min.

3.1.4. Information on the source material

3.1.4.1. Information related to the genetically modified microorganism

The glucose oxidase is produced with the genetically modified production strain *A. oryzae* NZYM-KP, which is deposited in the [redacted] with the deposit number [redacted].

3.1.4.2. Characteristics of the parental and recipient microorganisms

The *A. oryzae* recipient strain, [redacted], has been developed from the parental strain *A. oryzae* [redacted]. The taxonomic classification of both the parental and the recipient strain has been confirmed by [redacted]. The recipient strain [redacted] has a long history of use in the production of food enzymes. The strain was developed from the parental strain, [redacted]:

1) [redacted]
2) [redacted]
3) [redacted]
4) [redacted]

[redacted]

3.1.4.3. Characteristics of introduced sequences

The gene encoding the glucose oxidase, [redacted]

3.1.4.4. Description of the genetic modification process

[redacted] A clone showing increased expression of glucose oxidase was selected as the production strain (NZYM-KP).

3.1.4.5. Safety aspects of the production strain

[redacted]

The production strain NZYM-KP differs from the recipient strain [redacted]

The genetic modifications do not raise safety concerns.

3.1.5. Manufacturing process

The food enzyme is manufactured according to Food Hygiene Regulation (EC) No 852/2004[5], with food safety procedures based on Hazard Analysis and Critical Control Points (HACCP), and in accordance with Good Manufacturing Practice (GMP).

The food enzyme is produced by a pure culture in a contained, [redacted], [redacted] fermentation system with conventional process controls in place.

After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration. The filtrate containing the enzyme is then further purified and concentrated by [redacted].

After stabilisation with [redacted], the food enzyme preparation is commercialised as a liquid and solid product.

The production strain could not be detected in [redacted]

No DNA from the production strain was detected in [redacted]

The Panel considered the information provided on the raw materials and the manufacturing process as to be sufficient.

3.1.6. Safety for the environment

The production strain and its DNA were not detected in the final product. Therefore, the Panel concluded that there is no safety concern for the environment.

3.1.7. Case of need and intended conditions of use

The original uses proposed by the applicant were baking and cereal-based processes. In the course of the evaluation process, the applicant informed EFSA about withdrawal of the intended use in cereal based processes.

The resulting intended use is in baking processes at recommended use level up to 1,000 GODU/kg flour, corresponding to 13.9 mg TOS/kg flour.

In baking processes, glucose oxidase is used to increase the strength of the dough and facilitate its handling. The food enzyme is added during mixing of the raw materials.

[5] 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, p. 3–21.

3.1.8. Reaction and fate in food

The glucose oxidase catalyses the oxidation of β-D-glucose to D-glucono-δ-lactone and hydrogen peroxide using molecular oxygen as acceptor of electrons.

The D-glucono-δ-lactone is permitted as a carrier in food enzymes.[6]

Hydrogen peroxide is considered to be the active agent responsible for the intended function of glucose oxidase in dough preparation. Hydrogen peroxide reinforces the gluten network via oxidation of cysteine residues and the resulting formation of disulfide bonds (Bonet et al., 2006). In addition, hydrogen peroxide has been shown to induce the formation of dityrosine cross links through oxidative coupling of tyrosine residues in gluten proteins (Tilley et al., 2001; Takasaki et al., 2005). These reactions naturally occur during the preparation of dough; the use of the glucose oxidase is intended to promote these reactions, and thereby improve and/or standardise the rheological properties of the dough. Although the food enzyme contains catalase, which degrades hydrogen peroxide in the dough, the applicant demonstrated that the activity present was insufficient to affect the levels of hydrogen peroxide produced by the action of the glucose oxidase.

The glucose oxidase is specific in its action under the intended conditions of use and is not known to catalyse other reactions than this oxidation of glucose leading to the formation of hydrogen peroxide and D-glucono-1,5-lactone. Concerning the reactions of hydrogen peroxide in the dough, there is no expectation of oxidation products other than those normally formed during the dough-making and baking processes.

According to the data provided on the thermostability, it is anticipated that the glucose oxidase is inactivated during baking processes under the conditions of use.

3.2. Dietary exposure

Dietary exposure estimates were calculated using the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016). The assessment of the food processes covered in this opinion involved selection of relevant food groups and application of process and technical conversion factors (Appendix B). These input data were subject to a stakeholder consultation through open calls,[7] and adjusted in accordance with feedback received.

3.2.1. EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (hereinafter the EFSA Comprehensive Database[8]) has been populated with detailed national data on food consumption. Competent authorities in European countries provide EFSA with data regarding the level of food consumption by individual consumers, as taken from the most recent national dietary survey in their country (EFSA, 2011a).

The food consumption data gathered by EFSA were collected using different methodologies and thus direct country-to-country comparisons should be made with caution. Depending on the food category and the level of detail used in exposure calculations, uncertainties might be introduced owing to subjects possibly underreporting and/or misreporting of consumption amounts. Nevertheless, the EFSA Comprehensive Database is the best available source of food consumption data across Europe.

Food consumption data from the following population groups: infants, toddlers, children, adolescents, adults and the elderly were used for the exposure assessment. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Appendix A).

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b).

3.2.2. Exposure assessment methodology

Chronic exposure was calculated based on individual consumption, averaged over the total survey period, excluding surveys with only one day per subject. High-level exposure/intake was calculated for

[6] Annex III Part 3 of the Reg. (EU) No 1130/2011 amending Annex III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives by establishing a Union list of food additives approved for use in food additives, food enzymes, food flavourings and nutrients.

[7] http://www.efsa.europa.eu/en/data/call/161110

[8] http://www.efsa.europa.eu/en/food-consumption/comprehensive-database

only those population groups, in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011a).

The exposure per FoodEx category was subsequently added to derive an individual total exposure per day. Finally, these exposure estimates were averaged over the number of survey days and normalised for individual body weight (bw), resulting in an individual average exposure/day per kg bw for the survey period. This was done for all individuals in the survey and per age class, resulting in distributions of individual average exposure per survey and age class. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class.

3.2.3. Exposure to food enzyme–TOS according to the intended use proposed by the applicant

Dietary exposure to the food enzyme–TOS was based on intended use and the recommended maximum use levels of the food enzyme–TOS provided by the applicant (Table 2). Food enzyme–TOS exposure was calculated from foods produced involving a baking process.

Relevant food groups and/or individual foods were selected from the Comprehensive Database and were assumed to always contain the food enzyme–TOS at the maximum recommended use level. This will result in an overestimation of exposure to food enzyme–TOS.

To facilitate matching of the reported use levels for baking processes with foods identified in the Comprehensive Database, the selected foods were disaggregated to ingredient level as appropriate, and converted into the corresponding raw material, i.e. flour, via the application of conversion factors (Appendix B). For example, consumption of 100 g of bread was converted into an intake of 70 g flour (recipe fraction of 0.7) and then multiplied by 1.17 mg TOS/kg flour, as provided by the applicant, to arrive at an exposure of 0.08 mg TOS/100 g bread.

Dietary exposure to the food enzyme–TOS was calculated by multiplying values reported for each food category by their respective consumption amount per kilogram of body weight separately for each individual in the database. Table 2 provides an overview of the derived exposure estimates. The average and 95th percentile exposure to the food enzyme–TOS per age class, country and survey are reported in Appendix C – Table 1. The contribution of the food enzyme–TOS from each FoodEx category to the total dietary exposure is indicated in Appendix C – Table 2.

Table 2: Summary of estimated dietary exposure to food enzyme–TOS in six population groups

Population group	Estimated exposure (mg/kg bw 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)	0.010–0.042 (6)	0.036–0.088 (10)	0.038–0.083 (18)	0.023–0.055 (17)	0.017–0.034 (17)	0.016–0.029 (14)
Min.–max. 95th percentile (number of surveys)	0.057–0.119 (5)	0.083–0.148 (7)	0.071–0.156 (18)	0.041–0.109 (17)	0.032–0.066 (17)	0.030–0.053 (14)

bw: body weight.

3.2.4. 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
	Exposure to food enzyme–TOS
Model input data	
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/–
Use of data from food consumption survey 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 likely to contain the food enzyme	+/–
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 a considerable overestimation of the exposure.

3.3. Toxicological data

A battery of toxicological tests including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian chromosomal aberration test and a repeated dose 90-day oral toxicity study in rats has been provided. The batch 4 (Table 1) which is used for toxicological testing has lower specific activity compared to the batches used for commercialisation, and thus is considered cruder and suitable for toxicological testing.

The principal enzyme activity of the tested material is glucose oxidase, producing hydrogen peroxide (cytotoxic and mutagenic compound *in vitro*) in the presence of glucose. Therefore, in the genotoxicity tests, the glucose oxidase was inactivated by heat treatment for 30 min at 60°C at pH 2 and subsequently adjusted to neutral pH. Considering the fact that the food enzyme is intended to be used in baking processes, the Panel decided that this inactivation is representative for the food enzyme as consumed.

3.3.1. Genotoxicity

3.3.1.1. Bacterial reverse mutation test

In order to investigate the potential of the food enzyme to induce gene mutations, a bacterial reverse mutation assay (Ames test) was performed according to OECD Test Guideline 471 (OECD, 1997a) and following Good Laboratory Practice (GLP) in four strains of *Salmonella* Typhimurium (TA1535, TA100, TA1537 and TA98) and *E. coli* WP2uvrA. The test was performed in the presence or absence of metabolic activation applying the 'treat and plate assay' for *Salmonella* Typhimurium and the direct plate incorporation assay for *E. coli*. Two experiments were carried out in triplicate plating using six concentrations of the food enzyme (156, 313, 625, 1,250, 2,500 and 5,000 μg dry matter/plate, corresponding to 127, 256, 511, 1,021, 2,042 and 4,084 μg TOS/plate), using appropriate positive controls and sterile water as a negative control. All positive control chemicals induced significant increases in revertant colony numbers, confirming the sensitivity of the tests and the efficacy of the S9-mix. No toxicity was observed at any dose level of the test substance. However, growth stimulation was observed in most of the tested conditions after treatment with the food enzyme. This increase did not result in a noticeable increase in the corresponding levels of revertants.

Upon treatment with the food enzyme, the numbers of the revertant colonies were comparable to the values observed in the vehicle control groups in any tester strain, both in the presence and absence of metabolic activation. The Panel concluded that the food enzyme did not induce gene mutations in the bacterial reverse mutation assay under the test conditions employed for this study.

3.3.1.2. *In vitro* mammalian chromosome aberration test

The *in vitro* chromosome aberration test was carried out according to the OECD Test Guideline 473 (OECD, 1997b) and following GLP. Whole blood cultures were treated with purified water (negative control), the food enzyme or appropriate positive controls both in the presence and absence of metabolic activation. Based on the results of a preliminary cytotoxicity assay performed in a range of concentrations from 38 to 5,000 μg food enzyme/mL, the cells were treated with 3,441, 4,301 and 5,376 μg food enzyme/mL (corresponding to 368, 460 and 575 μg TOS/mL) applying a short-term treatment (3 h followed by 17 h recovery) in the presence and absence of S9-mix. The highest concentration induced approximately 32% and 0% reduction in mitotic index in the absence and presence of S9, respectively. In the second experiment, the concentrations of the food enzyme tested were 501, 1,187 and 2,109 μg food enzyme/mL (corresponding to 54, 127 and 226 μg TOS/mL) in a continuous treatment (20+0 h) without S9, and 3,613, 4,250 and 5,000 μg food enzyme/mL (corresponding to 387, 455 and 535 μg TOS/mL) for the short treatment in the presence of S9-mix. The highest concentrations induced approximately 49% and 0% reduction in mitotic index in the absence and presence of S9, respectively. Two hundred metaphases per experimental point were analysed. The Panel noted inconsistencies in the data reporting between the summary tables and the detailed presentation of type and number of chromosomal aberrations observed. Overall, no statistically significant increase in the frequency of chromosomal aberrations was observed in the treated cultures compared to the negative controls. One exception was the intermediate concentration tested (4,301 μg food enzyme/mL) in the short-term treatment in the presence of S9-mix, showing a frequency of aberrations above the historical vehicle control range; this increase was not dose-related, not statistically significant and not reproducible, and thus, it was considered not biologically relevant. In addition, the Panel noted a value above the observed range of the vehicle controls also at the mid-dose tested in the short-term treatment without S9-mix (4,301 μg food enzyme/mL). The increase was not dose-related or statistically significant, therefore was not considered of biological relevance. The frequencies of cells with numerical aberrations fell within the historical negative control ranges in all tested conditions. The Panel concluded that the food enzyme did not induce chromosomal aberration in cultured human peripheral blood lymphocytes when tested under the experimental conditions employed for this study.

3.3.2. Repeated dose 90-day oral toxicity study in rodents

A repeated dose 90-day oral toxicity study was performed according to OECD Test Guideline 408 (OECD, 1998) and following good laboratory practice (GLP). Four groups of 10 male and 10 female specific-pathogen-free Sprague–Dawley (Crl:CD(SD)IGS BR) rats were given the food enzyme by gavage at dose levels of 114, 341 or 1,135 mg TOS/kg bw per day. Controls received the vehicle (water).

There was one unscheduled death in the high-dose male group during week 9 of the study, probably due to misdosing.

In high-dose females, non-significant decreases in food consumption, body weight and body weight gain were observed. These changes were only slight (below 10% compared to controls) and are not considered to be adverse.

In the FOB tests for neurotoxicity (performed from week 1 to week 13), occasional deviations from the control were observed. These sporadic changes did not show a dose-response relationship and were only seen at single time-points and therefore they were not considered to be adverse.

In haematology, a statistically significant increase in prothrombin time was observed in the high-dose males. This was due to the high value in one animal and therefore not considered to be of toxicological concern. In the low- and mid-dose males, statistically significant increases in neutrophils associated with a non-significant increase in white blood cell counts were observed. No differences were observed in females. A statistically significant increase in mean absolute spleen weight was observed in the low- and mid-dose males without any pathological changes. As this increase was slight, and no dose-related, this effect was not considered relevant.

In clinical chemistry, the values for which significant differences were observed were sporadic, not dose-related and were small in magnitude. The effects were considered to be of no toxicological relevance.

At necropsy and histopathology, evaluation of dark (3/10) and spongy lung lobes (2/10) and/or subacute interstitial pneumonia in high-dose males (2/10) was observed. One of these animals also had oesophageal myositis. The 90-day study authors ascribe these lung effects to the gavage-dosing procedure, but it is noteworthy that both occurred only in the high dose and not at any other dose level or in the females. In addition, the intercurrent death (i.e. animal 37) had the same type of lung damage. In the absence of better evidence that these lung effects are indeed the result of gavage misdosing, these observations should be taken into account.

No other significant effects were observed.

Overall, the Panel derived a no-observed-adverse-effect level (NOAEL) based on the mid-dose level of 341 mg TOS/kg bw per day.

The NOAEL derived from 341 mg TOS/kg bw per day was compared to the exposure estimates of 0.010–0.088 mg/kg bw per day at the mean and from 0.030 to 0.156 mg TOS/kg bw per day at the 95th percentile, resulted in a margin of exposure (MOE) above 2,186, indicating that there is no safety concern.

3.4. 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 glucose oxidase produced with the genetically modified *A. oryzae* strain NZYM-KP was assessed by comparison of its amino acid sequence with those of known allergens according to the 'EFSA 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 window of 80 amino acids as the criterion, one match was found. The matching allergen was Mala s 12 from *Malassezia sympodialis* (formerly known as *Pityrosporum*), which is a ubiquitous component of the human skin microbiome. Mala s 12 has sequence similarity with the glucose-methanol-choline oxidoreductase enzyme superfamily (Zargari et al., 2007), which also includes glucose oxidase. Mala s 12 is a known contact allergen that can induce IgE- and T-cell-mediated allergic reaction in atopic eczema patients characterised by an impaired skin barrier. Considering that oral allergic reactions are mediated by IgE, elicitation reactions upon dietary exposure to this food enzyme cannot be excluded but as the yeast that expresses this allergen is an ubiquitous component of the skin microflora, the likelihood of such elicitation reactions to occur after oral exposure through food is considered to be low.

No oral or respiratory allergic reactions to this glucose oxidase have been reported.

The applicant provided a study by Bindlev-Jensen et al. (2006) who investigated the possible cross reactivity of 19 different commercial food enzymes in allergic patients (400 patients allergic to inhalation allergens, food allergens, allergens of bee or wasp). Glucose oxidase from a genetically modified *A. oryzae* donor organism was positive in the skin prick test in three patients. The enzyme was further tested by ingestion (double-blind, placebo-controlled food challenge (DBPCFC)) and found to be negative to both active and placebo challenges. Despite the fact that no allergic reactions have been observed in these individuals, no conclusion can be drawn since the amino acid sequences of the allergens to which the patients were sensitised are not known.

The Panel considers that under the intended condition 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 occurring is considered to be low.

Conclusions

Based on the microbial source, the genetic modifications, the manufacturing process, the compositional, biochemical, toxicological data and the dietary exposure assessment, the Panel concluded that the food enzyme glucose oxidase produced with the genetically modified *A. oryzae* strain NZYM-KP does not give raise to safety concerns under the intended conditions of use.

Regarding the allergenicity assessment, 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 can be considered to be low.

Documentation provided to EFSA

1) Dossier 'Glucose oxidase from a genetically modified strain of *Aspergillus oryzae* (strain NZYM-KP)'. August 2013. Submitted by Novozymes A/S.
2) Additional information submitted on 3 September 2014 by the applicant.
3) Additional information submitted in October 2017 by the applicant.

References

Bindlev-Jensen C, Skov PS, Roggen EL, Hvass P and Brinch DS, 2006. Investigation on possible allergenicity of 19 different commercial enzymes used in the food industry. Food and Chemical Toxicology 44, 1909–1915.

Bonet A, Rosell CM, Caballero PA, Gómez M, Pérez-Munuera I and Lluch MA, 2006. Glucose oxidase effect on dough rheology and bread quality: a study from macroscopic to molecular level. Food Chemistry, 99, 408–415. https://doi.org/10.1016/j.foodchem.2005.07.043

[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), 2009. 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), 2011a. 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 (European Food Safety Authority), 2011b. Evaluation of the FoodEx, the food classification system applied to the development of the Comprehensive European Food Consumption Database. EFSA Journal 2011;9(3):1970, 27 pp. https://doi.org/10.2903/j.efsa.2011.1970

EFSA CEF Panel (EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids), 2009. Guidance of the Scientific Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) on the Submission of a Dossier on Food Enzymes for Safety Evaluation by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids. EFSA Journal 2009;1305, 1–26. https://doi.org/10.2903/j.efsa.2009.1305. Available online: https://www.efsa.europa.eu/en/efsajournal/pub/1305

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), Silano V, Bolognesi C, Castle L, Cravedi JP, Fowler P, Franz R, Grob K, Gürtler R, Husøy T, Kärenlampi S, Mennes W, Milana MR, Penninks A, Smith A, Tavares Poças MF, Tlustos C, Wölfle D, Zorn H, Zugravu CA, Arcella D, Liu Y and Engel KH, 2016. Panel statement on the exposure assessment of food enzymes. EFSA Journal 2016; 10(10):4581, 11 pp. https://doi.org/10.2903/j.efsa.2016.4581

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. Available online: http://www.efsa.europa.eu/en/efsajournal/pub/1700.htm

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, 63–67. Available online: ftp://ftp.fao.org/docrep/fao/009/a0675e/a0675e00.pdf

OECD (Organisation for Economic Cooperation and Development), 1997a. Bacterial Reverse Mutation Test. Guideline 471, adopted 21.7.1997. Available online: http://www.oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en;jsessionid=9zfgzu35paaq.x-oecd-live-01

OECD (Organisation for Economic Cooperation and Development), 1997b. *In vitro* Mammalian Chromosomal Aberration Test. Guideline 473, adopted 21.7.1997. Available online: http://www.oecd-ilibrary.org/environment/test-no-473-in-vitro-mammalian-chromosome-aberration-test_9789264071261-en

OECD (Organisation for Economic Cooperation and Development), 1998. Repeated Dose 90-day Oral Toxicity Study in Rodents. Guideline 408, adopted 21.9.1998. Available online: http://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en

Takasaki S, Kato Y, Murata M, Homma S and Kawakishi S, 2005. Effects of peroxidase and hydrogen peroxide on the dityrosine formation and the mixing characteristics of wheat-flour dough. Bioscience, Biotechnology, and Biochemistry 69, 1686–1692. https://doi.org/10.1271/bbb.69.1686

Tilley KA, Benjamin RE, Bagorogoza KE, Okot-Kotber BM, Prakash O and Kwen H, 2001. Tyrosine cross-links: molecular basis of gluten structure and function. Journal of Agriculture and Food Chemistry 49, 2627–2632. https://doi.org/10.1021/jf010113h

Zargari A, Selander C, Rasool O, Ghanem M, Gadda G, Crameri R and Scheynius A, 2007. Mala s 12 is a major allergen in patients with atopic eczema and has sequence similarities to the GMC oxidoreductase family. Allergy, 62, 695–703. https://doi.org/10.1111/j.1398-9995.2006.01291.x

Abbreviations

ABTS	2,2-azino-di-(3-ethylbenzthiazoline)-6-sulfonate
bw	body weight
CAS	Chemical Abstracts Service
CFU	colony forming units
DBPCFC	double-blind, placebo-controlled food challenge
[redacted]	[redacted]
EINECS	European Inventory of Existing Commercial Chemical Substances
GLP	good laboratory practice
GMM	genetically modified microorganism
GMP	Good Manufacturing Practice
GODU	Glucose Oxidase Units
HACCP	Hazard Analysis and Critical Control Points
IgE	immunoglobulin E
[redacted]	[redacted]
IUBMB	International Union of Biochemistry and Molecular Biology
MOE	margin of exposure
NOAEL	no-observed-adverse-effect level
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
QPS	qualified presumption of safety
SDS-PAGE	sodium dodecyl sulfate-poly acrylamide gel electrophoresis
TOS	total organic solids

Appendix A – Population groups considered for the exposure assessment

Population	Age range	Countries with food consumption surveys covering more than one day
Infants	From 12 weeks up to and including 11 months of age	Bulgaria, Denmark, Finland, Germany, Italy, United Kingdom
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Finland, Germany, Italy, Netherlands, Spain, United Kingdom
Children[(a)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden, United Kingdom
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, Spain, Sweden, United Kingdom
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain, Sweden, United Kingdom
The elderly[(a)]	From 65 years of age and older	Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Romania, 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, 2011a).

Appendix B – FoodEx categories used to derive exposure estimates for the food enzyme–TOS and the respective conversion factors

FoodEx code	FoodEx category	Conversion factor from FoodEx food group to raw material[a]	Recipe fraction[b]	mg TOS/kg flour
A.01	Grains and grain-based products (unspecified)	0.8	1	13.9
A.01.03	Grain milling products (unspecified)	1	1	13.9
A.01.03.001	Wheat milling products (unspecified)	1	1	13.9
A.01.03.001.001	Wheat flour, brown	1	1	13.9
A.01.03.001.002	Wheat flour, Durum	1	1	13.9
A.01.03.001.003	Wheat flour, white	1	1	13.9
A.01.03.001.004	Wheat flour, wholemeal	1	1	13.9
A.01.03.001.005	Graham flour	1	1	13.9
A.01.03.001.006	Wheat flour, gluten free	1	1	13.9
A.01.03.001.014	Wheat starch	1.2	1	13.9
A.01.03.002	Rye milling products (unspecified)	1	1	13.9
A.01.03.002.001	Rye flour, gluten free	1	1	13.9
A.01.03.002.002	Rye flour, light	1	1	13.9
A.01.03.002.003	Rye flour, medium	1	1	13.9
A.01.03.002.004	Rye flour, wholemeal	1	1	13.9
A.01.03.003	Buckwheat milling products (unspecified)	1	1	13.9
A.01.03.003.001	Buckwheat flour	1	1	13.9
A.01.03.004	Corn milling products (unspecified)	1	1	13.9
A.01.03.004.001	Corn flour	1	1	13.9
A.01.03.004.003	Corn starch	1.3	1	13.9
A.01.03.005	Oat milling products (unspecified)	1	1	13.9
A.01.03.005.002	Oat flour	1	1	13.9
A.01.03.005.004	Oat starch	1.2	1	13.9
A.01.03.006	Rice milling products (unspecified)	1	1	13.9
A.01.03.006.001	Rice flour	1	1	13.9
A.01.03.006.002	Rice flour, white	1	1	13.9
A.01.03.006.003	Rice flour, instant	1	1	13.9
A.01.03.006.004	Rice starch	1.2	1	13.9
A.01.03.007	Spelt milling products	1	1	13.9
A.01.03.008	Other milling products (unspecified)	1	1	13.9
A.01.03.008.001	Amaranth flour	1	1	13.9
A.01.03.008.002	Barley flour	1	1	13.9
A.01.03.008.003	Chapatti flour	1	1	13.9
A.01.03.008.004	Flour mix, wheat/rye/barley/oats	1	1	13.9
A.01.03.008.005	Millet flour	1	1	13.9
A.01.03.008.007	Sorghum flour	1	1	13.9
A.01.04	Bread and rolls (unspecified)	1	0.7	13.9
A.01.04.001	Wheat bread and rolls	1	0.7	13.9
A.01.04.002	Rye bread and rolls	1	0.7	13.9
A.01.04.003	Mixed wheat and rye bread and rolls	1	0.7	13.9
A.01.04.004	Multigrain bread and rolls	1	0.7	13.9
A.01.04.005	Unleavened bread, crisp bread and rusk (unspecified)	1	0.8	13.9
A.01.04.005.001	Crisp bread, rye wholemeal	1	0.9	13.9
A.01.04.005.002	Crisp bread, rye, light	1	0.9	13.9

FoodEx code	FoodEx category	Conversion factor from FoodEx food group to raw material[a]	Recipe fraction[b]	mg TOS/kg flour
A.01.04.005.003	Crisp bread, wheat, wholemeal	1	0.9	13.9
A.01.04.005.004	Crisp bread, wheat, light	1	0.9	13.9
A.01.04.005.005	Rusk, light	1	0.9	13.9
A.01.04.005.006	Rusk, wholemeal	1	0.9	13.9
A.01.04.005.007	Pita bread	1	0.7	13.9
A.01.04.005.008	Matzo	1	0.9	13.9
A.01.04.005.009	Tortilla	1	0.7	13.9
A.01.04.006	Other bread	1	0.7	13.9
A.01.04.007	Bread products	1	0.7	13.9
A.01.07	Fine bakery wares (unspecified)	1	0.5	13.9
A.01.07.001	Pastries and cakes (unspecified)	1	0.5	13.9
A.01.07.001.001	Beignets	1	0.15	13.9
A.01.07.001.002	Buns	1	0.7	13.9
A.01.07.001.003	Cake from batter	1	0.25	13.9
A.01.07.001.004	Cheese cream cake	1	0.24	13.9
A.01.07.001.005	Cheese cream sponge cake	1	0.24	13.9
A.01.07.001.006	Chocolate cake	1	0.24	13.9
A.01.07.001.007	Chocolate cake with fruits	1	0.24	13.9
A.01.07.001.008	Cream cake	1	0.24	13.9
A.01.07.001.009	Cream cheese cake	1	0.24	13.9
A.01.07.001.010	Cream custard cake	1	0.24	13.9
A.01.07.001.011	Cream custard sponge cake	1	0.24	13.9
A.01.07.001.012	Croissant	1	0.5	13.9
A.01.07.001.013	Croissant, filled with chocolate	1	0.5	13.9
A.01.07.001.014	Croissant, filled with cream	1	0.5	13.9
A.01.07.001.015	Croissant, filled with jam	1	0.5	13.9
A.01.07.001.016	Croquembouche	1	0.15	13.9
A.01.07.001.017	Doughnuts	1	0.24	13.9
A.01.07.001.018	Éclair	1	0.15	13.9
A.01.07.001.019	Flan	1	0.5	13.9
A.01.07.001.020	Fruit cake	1	0.6	13.9
A.01.07.001.021	Fruit pie	1	0.15	13.9
A.01.07.001.022	Cheese pie	1	0.15	13.9
A.01.07.001.023	Fruit tart	1	0.15	13.9
A.01.07.001.024	Gingerbread	1	0.6	13.9
A.01.07.001.025	Gougere	1	0.15	13.9
A.01.07.001.026	Kringles	1	0.25	13.9
A.01.07.001.027	Nut cream cake	1	0.24	13.9
A.01.07.001.028	Pancakes	1	0.25	13.9
A.01.07.001.029	Profiterole	1	0.15	13.9
A.01.07.001.030	Pyramid cake	1	0.25	13.9
A.01.07.001.031	Rhubarb flan	1	0.15	13.9
A.01.07.001.032	Scone	1	0.5	13.9
A.01.07.001.033	Sponge dough	1	0.25	13.9
A.01.07.001.034	Sponge cake	1	0.25	13.9
A.01.07.001.035	Sponge cake roll	1	0.25	13.9
A.01.07.001.036	Muffins	1	0.25	13.9

FoodEx code	FoodEx category	Conversion factor from FoodEx food group to raw material[(a)]	Recipe fraction[(b)]	mg TOS/kg flour
A.01.07.001.037	Waffles	1	0.25	13.9
A.01.07.001.038	Apple strudel	1	0.15	13.9
A.01.07.001.039	Cream-cheese strudel	1	0.24	13.9
A.01.07.001.040	Cheese pastry goods from puff pastry	1	0.15	13.9
A.01.07.001.041	Croissant from puff pastry	1	0.6	13.9
A.01.07.001.042	Brioche	1	0.5	13.9
A.01.07.001.044	Lebkuchen	1	0.6	13.9
A.01.07.001.045	Dumpling	1	0.5	13.9
A.01.07.001.046	Cake marbled, with chocolate	1	0.5	13.9
A.01.07.001.047	Marzipan pie	1	0.25	13.9
A.01.07.001.048	Baklava	1	0.15	13.9
A.01.07.002	Biscuits (cookies)	1	0.9	13.9
A.01.07.002.001	Biscuits, sweet, plain	1	0.9	13.9
A.01.07.002.002	Biscuits, chocolate filling	1	0.81	13.9
A.01.07.002.003	Biscuits, cream filling	1	0.81	13.9
A.01.07.002.004	Biscuits, fruit filling	1	0.81	13.9
A.01.07.002.005	Biscuits, vanilla filling	1	0.81	13.9
A.01.07.002.006	Butter biscuits	1	0.81	13.9
A.01.07.002.007	Biscuit, iced	1	0.81	13.9
A.01.07.002.008	Speculaas	1	0.9	13.9
A.01.07.002.009	Biscuits, sweet, wheat wholemeal	1	0.9	13.9
A.01.07.002.010	Biscuits, oat meal	1	0.9	13.9
A.01.07.002.011	Biscuits, spelt meal	1	0.9	13.9
A.01.07.002.012	Biscuits, salty	1	0.9	13.9
A.01.07.002.013	Biscuits, salty, with cheese	1	0.81	13.9
A.01.07.002.014	Sticks, salty	1	0.81	13.9
A.17.03.003	Biscuits, rusks and cookies for children	1	0.9	13.9
A.18.04.001	Find bakery products for diabetics	1	0.5	13.9
A.19.01.001	Sandwich and sandwich-like meal	1	0.32	13.9
A.19.01.002	Pizza and pizza-like pies	1	0.3	13.9

TOS: total organic solids.
(a): Available online: http://www.fao.org/fileadmin/templates/ess/documents/methodology/tcf.pdf
(b): Derived from publically available recipe information, and/or food label information (such as Mintel's Global New Products Database http://www.mintel.com/global-new-products-database).

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

Information for this appendix is provided in an Excel file (downloadable https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2018.5319#efs25319-sup-0001).

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: The contribution of the food enzyme–TOS from each FoodEx category to the total dietary exposure.