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# Safety evaluation of the food enzyme maltogenic amylase from a genetically modified *Bacillus subtilis* (strain NZYM-SM)

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## Abstract

The food enzyme considered is a maltogenic amylase (glucan 1,4-α-maltohydrolase; EC 3.2.1.133) produced with the genetically modified *Bacillus subtilis* strain NZYM-SM by Novozymes A/S. The food enzyme contains neither the production organism nor recombinant DNA. The maltogenic amylase is intended for use in baking processes and starch processing for glucose syrups production. Based on the maximum use levels recommended for the food processes 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 0.168 mg TOS/kg body weight (bw) per day in European populations. The food enzyme did not induce gene mutations in bacteria or chromosomal 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 (NOAEL) was derived (320 mg TOS/kg bw per day), which, compared with the dietary exposure, results in a sufficiently high margin of exposure. The allergenicity was evaluated by searching for similarity of the amino acid sequence to those of known allergens. Three matches to occupational respiratory allergens were found, however, the Panel considered that there are no indications for food allergic reactions to the food enzyme. Based on the genetic modifications performed, the manufacturing process, the compositional and biochemical data provided, the dietary exposure assessment, the findings in the toxicological studies and allergenicity assessment, the Panel concluded that the food enzyme maltogenic amylase from *Bacillus subtilis* strain NZYM-SM does not give rise to safety concerns under the intended conditions of use.



**Keywords:** food enzyme, glucan 1, 4-α-maltohydrolase, EC 3.2.1.133, maltogenic amylase, *Bacillus subtilis*, genetically modified microorganism

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# 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 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 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;
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 an approval via a Union list.

The 'Guidance on submission of a dossier on a food enzyme for 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 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.

Five applications have been introduced by the companies 'Novozymes A/S', 'AB Enzymes GmbH'. 'Ajinomoto Europe SAS' and 'Nagase (Europa) GmbH' for the authorisation of the food enzymes Beta-galactosidase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-BT), Mannan endo-1,4-beta-mannosidase (β-mannanase) from a genetically modified strain of *Trichoderma reesei* (strain RF6232), Transglutaminase from *Streptoverticillium mobaraense* (strain S-8112), Maltogenic amylase from a genetically modified strain of *Bacillus subtilis* (strain NZYM-SM) and Glucanase from *Streptomyces violaceoruber* (strain pGlu).

Following the requirements of Article 12.1 of Commission Regulation (EU) No 234/2011[2] implementing Regulation (EC) No 1331/2008[3], the Commission has verified that the five applications fall within the scope of the food enzyme Regulation and contains all the elements required under Chapter II of that Regulation.

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

[2] 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.

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

### 1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes Beta-galactosidase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-BT), Mannan endo-1,4-beta-mannosidase (β-mannanase) from a genetically modified strain of *Trichoderma reesei* (strain RF6232), Transglutaminase from *Streptoverticillium mobaraense* (strain S-8112), Maltogenic amylase from a genetically modified strain of *Bacillus subtilis* (strain NZYM-SM) and Glucanase from *Streptomyces violaceoruber* (strain pGlu) in accordance with the 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 request to carry out the safety assessment of the food enzyme Maltogenic amylase from a genetically modified strain of *Bacillus subtilis* (strain NZYM-SM).

## 1.3. Information on existing authorisations and evaluations

The applicant reports that the Danish and French authorities have evaluated and authorised the use of the food enzyme from a genetically modified *Bacillus subtilis* strain NZYM-SM in starch processing, baking processes and maltose syrup production.

# 2. Data and methodologies

## 2.1. Data

The applicant has submitted a dossier supporting the application for authorisation of the food enzyme maltogenic amylase from a genetically modified strain of *B. subtilis* (strain NZYM-SM).

## 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 Guidances from the EFSA Scientific Committee.

The current 'Guidance on the submission of a dossier for safety evaluation of a food enzyme' (EFSA CEF panel, 2009) has been followed for the evaluation of this 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: Glucan 1,4-α-maltohydrolase
Systematic name: 4-α-D-glucan α-maltohydrolase
Synonyms: Maltogenase
IUBMB No: EC 3.2.1.133
CAS No: 160611-47-2.

### 3.1.2. Chemical parameters

The maltogenic amylase produced with the genetically modified *Bacillus subtilis* strain NZYM-SM consists of a single polypeptide of 686 amino acids. The molecular mass, derived from the amino acid sequence, was calculated to be 75.2 kDa. The sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) gel analysis consistently showed one major protein band in all batches, migrated slightly below 66 kDa reference protein.

The food enzyme was tested for other enzyme activities, i.e. lipase, protease and glucoamylase, which were below the limits of quantification (LOQ) of the applied assays, except for one commercial

batch with a very low amount of glucoamylase activity. No other enzyme activities relevant to the intended uses were reported by the applicant.

Data on the chemical parameters of the food enzyme were provided for four food enzyme batches, three batches used for commercialisation and one batch used for the toxicological tests (Table 1). The average Total Organic Solids (TOS) content of the three food enzyme batches used for commercialisation was 4.4%; the values ranged from 2.9% to 5.3% (Table 1).

The average enzyme activity/TOS ratio of the three food enzyme batches used for commercialisation was 330 Maltogenic Amylase Novo Units MANU/mg TOS; the values ranged from 273 to 362 MANU/mg TOS (Table 1). The average activity/TOS ratio of 330 MANU/mg TOS was used for subsequent calculations.

**Table 1:** Compositional data provided for the food enzyme

| Parameter | Unit | Batch | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4[(a)] |
| Maltogenic amylase activity | MANU/g batch[(b)] | 19,200 | 10,300 | 13,400 | 8,600 |
| Protein | % | 4.9 | 2.9 | 4.7 | ND |
| Ash | % | 1.0 | 0.8 | 1.1 | 4.8 |
| Water | % | 93.7 | 96.3 | 94.0 | 86.1 |
| Total organic solids (TOS)[(c)] | % | 5.3 | 2.9 | 4.9 | 9.1 |
| Activity/mg TOS | MANU/mg TOS | 362 | 355 | 273 | 94.5 |

ND: not determined.
(a): Batch used for the toxicological studies.
(b): MANU: Maltogenic Amylase Novo 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 were below the limits of detection of the employed methodologies.

No antimicrobial activity was detected in any of these batches (FAO/WHO 2006).

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 identity 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 maltogenic amylase catalyses the hydrolysis of (1→4)-α-D-glucosidic linkages in amylose, amylopectin and related glucose polymers, liberating maltose units from the non-reducing end of the polymer chain.

The enzymatic activity is determined on the basis of an in-house method using a maltotriose standard and expressed in Maltogenic Amylase Novo Units/g (MANU/g). One MANU is defined as the amount of enzyme that produces 1 μmol glucose per minute using maltotriose as substrate under the defined assay conditions (reaction conditions: pH = 5.0, T = 37°C, incubation time 30 min). The enzymatic hydrolysis of maltotriose results in the release of glucose, which is determined quantitatively using a glucose hexokinase assay.

The food enzyme has been characterised regarding its activity depending on temperature and pH. The temperature profile of the food enzyme was measured from 40°C to 100°C. The maltogenic amylase is active at temperatures below 90°C with an optimum between 60°C and 70°C at pH 5.5. The activity is completely lost after incubation of the enzyme for 30 min at 90°C. The pH optimum is around pH 5 at 30°C.

### 3.1.4. Information on the source material

#### 3.1.4.1. Information relating to the genetically modified microorganism

The maltogenic amylase production strain *Bacillus subtilis* NZYM-SM is deposited in the [redacted] with the deposit number [redacted].

#### 3.1.4.2. Characteristics of the parental and recipient microorganism

The parental microorganism is the bacterium *B. subtilis*, strain [redacted] ([redacted]).

The identity of the parental strain has been confirmed by whole genome sequencing ([redacted]). The parental strain has been tested for the absence of cytotoxicity in VERO cells. An intermediate strain has been tested both in CHO-K1 (Pedersen et al., 2002) and in VERO cells. Both proved negative.

The recipient strain, *B. subtilis* [redacted], has been developed from the parental strain [redacted]

#### 3.1.4.3. Characteristics of the donor organisms

[redacted]

#### 3.1.4.4. Description of the genetic modification process

The production strain NZYM-SM was developed from the recipient strain [redacted]

[redacted]

#### 3.1.4.5. Safety aspects of the genetic modification

[redacted]

### 3.1.5. Manufacturing process

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[4] and in accordance with current Good Manufacturing Practice (GMP).

The food enzyme is produced by a pure culture in a contained, submerged, fed-batch fermentation system with conventional process controls in place. The identity and purity of the culture are checked at each transfer step from frozen vials to the end of fermentation.

The food enzyme produced is recovered from the fermentation broth after biomass separation using filter press filtration. Further purification and concentration involve a series of filtration steps, including ultrafiltration and sterile filtration.

The food enzyme is then formulated as a liquid or solid product.

The absence of the production microorganism in the food enzyme was demonstrated in [redacted]

No recombinant DNA was detected in three independent batches in triplicate [redacted]

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

### 3.1.6. Safety for the environment

The production strain and its recombinant DNA were not detected in the final product. Accordingly, no environmental risk assessment is required (EFSA GMO Panel, 2011).

### 3.1.7. Case of need and intended conditions of use

In the original submission, the intended uses of the food enzyme were: starch processing for glucose syrup production, baking, cereal based and brewing processes. In the course of the evaluation, the applicant informed EFSA about withdrawal of use of the food enzyme in cereal based and brewing processes.

The intended uses and the recommended use levels are summarised in Table 2.

[4] 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.

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

| Food manufacturing process[(a)] | Raw material | Recommended dosage of the food enzyme |
|---|---|---|
| Baking processes[(b)] | Flour | Up to 5,000 MANU/kg flour corresponding to 15 mg TOS/kg flour |
| Starch processing for glucose syrups production | Starch | Up to 16,500 MANU/kg starch corresponding to 49.5 mg TOS/kg starch |

MANU: Maltogenic Amylase Novo Units; TOS: Total Organic Solids.
(a): The description provided by the applicant has been harmonised by EFSA 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): Restricted to bread-making including buns, scones and brioches.

In baking processes, the food enzyme is added during the preparation of the dough. It is used to hydrolyse starch and related polysaccharides to delay the staling process.

In starch processing for glucose syrups production, the food enzyme is added during the saccharification step. It is used to degrade starch polysaccharides into maltose and glucose in an efficient way.

### 3.1.8. Reaction and fate in food

The maltogenic amylase catalyses the hydrolysis of (1→4)-α-D-glucosidic linkages in amylose, amylopectin and related glucose polymers, liberating maltose units from the non-reducing end of the polymer chain.

Experimental data on the removal (> 99%) of protein in the course of starch processing for glucose syrups production have been provided (Documentation provided to EFSA No 3). The Panel considered the evidence as sufficient to conclude that residual amounts of TOS are removed by the purification steps applied during the production of glucose syrups, i.e. filtration, ion exchange chromatography, carbon treatment, crystallisation.

The data and information provided indicate that the maltogenic amylase is inactivated during the baking processes under the intended conditions of use.

## 3.2. Dietary exposure

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 process 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,[5] 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 (hereafter the EFSA Comprehensive Database[6]) 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 population groups: infants, toddlers, children, adolescents, adults and the elderly were used for the exposure assessment. For the present assessment, food

[5] http://www.efsa.europa.eu/en/data/call/161110
[6] http://www.efsa.europa.eu/en/food-consumption/comprehensive-database

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 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

Exposure to the food enzyme–TOS was based on intended uses and the recommended maximum use levels of the food enzyme–TOS provided by the applicant (Table 2). Foods/ingredients derived through starch processing, i.e. glucose syrups, were excluded from the analysis, as the Panel considered the presence of residual amounts of TOS in glucose syrups as negligible (see Section 3.1.7). Therefore, food enzyme–TOS exposure was calculated from foods produced involving a baking process only. The applicant proposed a restricted number of baking processes (Table 2), however, the Panel decided to follow the exposure methodology described in Section 3.2, i.e. inclusion of all baking applications, since no need for refining the exposure was identified.

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 process 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 15.0 mg TOS/kg flour, as provided by the applicant, to arrive at an exposure of 1.05 mg TOS.

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 (kg bw) separately for each individual in the database. Table 3 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 3:** 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) | 0.010–0.046 (6) | 0.039–0.095 (10) | 0.041–0.090 (18) | 0.025–0.060 (17) | 0.018–0.036 (17) | 0.017–0.031 (14) |
| Min–max 95th percentile (number of surveys) | 0.061–0.129 (5) | 0.089–0.160 (7) | 0.077–0.168 (18) | 0.044–0.118 (17) | 0.035–0.071 (17) | 0.032–0.057 (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 4.

**Table 4:** 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** | |
| The estimation considered all food groups involving baking processes (e.g. breads and cakes) | + |
| 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

The batch used for toxicological testing is an enzyme concentrate without addition of additives or other standardisation or stabilisation ingredients. It has been produced in accordance with the methods used for commercial batches, but the concentration has been done by means of evaporation instead of by ultrafiltration. The evaporation step removes water and thereby relatively increases non-enzymatic organic matter compared to activity. Table 1 shows that the food enzyme batch 4 used for the toxicological assays has the lowest specific activity (enzyme activity/mg TOS), which indicates that it is less pure than the commercial batches and thus can be considered as a 'worst-case' situation. Consequently, on the basis of the data provided, batch 4 is considered cruder than the three batches for commercialisation and its use for toxicological testing is considered acceptable.

### 3.3.1. Genotoxicity

#### 3.3.1.1. Bacterial reverse mutation test

To investigate the potential of the maltogenic amylase to induce gene mutations in bacteria, a bacterial reverse mutation assay (Ames test) was performed according to OECD Test Guideline 471 (OECD, 1997a) and following Good Laboratory Practice (GLP). Four strains of *Salmonella* Typhimurium (TA1535, TA100, TA1537 and TA98) and *Escherichia coli* WP2uvrA were used in the presence or absence of metabolic activation with S9-mix, applying the direct plate incorporation method. Two separate experiments were carried out using six different concentrations (0, 156, 313, 625, 1,250, 2,500 and 5,000 μg dry matter/plate) of the food enzyme, appropriate positive control chemicals and deionised water as a negative control. The concentrations tested corresponded to ca. 0, 102, 205,

409, 818, 1,637 and 3,273 μg TOS/plate. All positive controls induced significant increases in revertant colony numbers, confirming the sensitivity of the tests and the efficacy of the S9-mix. Upon treatment with the food enzyme, bacteriotoxic effects were not observed in this study. Small non-reproducible increases in the number of colonies without dose relation were observed in few test series with S9 mix. These increases were not considered toxicologically relevant. Upon treatment with the food enzyme, there was no evidence of mutagenic activity of the food enzyme in this mutation test.

The Panel concluded that the food enzyme maltogenic amylase did not induce gene mutations in the bacterial reverse mutation assay under the test conditions employed in this study.

#### 3.3.1.2. *In vitro* mammalian chromosomal aberration test

The *in vitro* chromosome aberration test was carried out according to the OECD Test Guideline 473 (OECD, 1997b) and following GLP. Cultures of peripheral blood human lymphocytes were prepared from the pooled blood of three female donors. The lymphocytes, proliferation of which was stimulated with phytohaemagglutinin (PHA), were treated with the food enzyme, sterile water (negative control) or appropriate positive controls in the absence or the presence of the S9-mix. Two experiments were performed. In the first experiment, applying 3 + 17 h treatment, the cultures were exposed to the food enzyme at concentrations of 3,200, 4,000 and 5,000 μg food enzyme/mL (corresponding to ca 291, 364 and 455 μg TOS/mL) in the presence and absence of the S9-mix. In the second experiment, applying continuous 20 + 0 h treatment without metabolic activation, concentrations scored for the chromosome aberrations were 1,886, 2,219 and 3,071 μg food enzyme/mL (corresponding to ca 172, 202 and 279 μg TOS/mL). For the short-term treatment (3 + 17 h) with metabolic activation, concentrations of 3,613, 4,250 and 5,000 μg food enzyme/mL (corresponding to 329, 387 and 455 μg TOS/mL) were tested. Reductions in the mitotic index of 47% and 1% were observed at 3,071 and 5,000 μg food enzyme/mL in the long-term treatment without S9-mix and the second short-term treatment in the presence of the S9-mix, respectively. Only cells with 44–46 chromosomes were analysed for chromosome aberrations, polyploidy and endoreduplication. Two hundred metaphases were analysed at each concentration. For all food enzyme concentrations used, the frequency of cells with chromosomal aberrations was similar to that of negative controls (values of $p \leq 0.05$ were considered as significant), except for 4,250 μg of food enzyme/mL in the second experiment in pulse 3 + 17 h treatment in the presence of S9-mix. As this effect was not reproducible, not concentration related, and found in one culture only, it is not considered to be of biological relevance.

The Panel concluded that the food enzyme maltogenic amylase did not induce chromosome aberrations, polyploidy and or endoreduplication in cultured human peripheral blood lymphocytes when tested under the test conditions employed in this study.

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

A repeated dose 90-day oral toxicity study was performed in accordance with OECD Test Guideline 408 (OECD, 1998) and following GLP. Groups of 10 male and 10 female Sprague–Dawley rats received the food enzyme orally via gavage volume of 10 mL/kg bw per day, corresponding to 97, 320 or 968 mg TOS/kg bw per day (referred to as low, mid and high dose groups). The control group received the vehicle water.

Food consumption of treated females was sporadically higher than that of the control animals and total food consumption of the low- and mid-dose females was significantly higher than that of the controls. This was reflected in a tendency for mean body weights of all treated females to be higher than that of the control animals from approximately day 15 but these differences seemed to be due partly to the marked contribution of individual animals to the mean group values. There was no dosage-related response, and group mean body weight gain of the food enzyme groups was within 15% of the control value. Although the food consumption of all treated males tended to be below that of the control group and significantly in the last 2 weeks of dosage, no significant difference was seen either between the tested groups or in the weight gain of the animals. The differences in food consumption were considered not to be toxicologically relevant.

At termination of the study, the relative neutrophil count (% of white blood cells) of high-dose males and females and the absolute neutrophil count of high-dose males and mid-dose females were significantly higher than those of the control groups. Relative, but not absolute lymphocytes were decreased and relative, but not absolute monocytes were significantly increased in high-dose males. No dose dependency was observed and values were within historical controls. Fibrinogen was

marginally increased in all treated females but there was no effect on clotting time. The haematological changes were not considered of toxicological importance.

A few statistically significant differences in clinical chemistry parameters were seen between the control group and the treated groups. The effects included increased urea and phosphorus in high-dose males, increased AST in high-dose females and increased beta protein in all treated females, but without a clear dose–response relationship.

Significant differences were seen between the control groups and the treated groups in urinalysis. The effects were increased epithelial cells in low- and high-dose males, increased *N*-acetyl-β-D-glucosaminidase (NAG)/mmol creatinine in high-dose females, increased leucocytes and epithelial cells in mid-dose females. The relative kidney weight of high-dose males and the absolute kidney weight of high-dose females were significantly higher than those of the control groups. Although no histopathological findings were observed at necropsy, the Panel decided as a conservative approach to select the mid-dose of 320 mg TOS/kg bw per day as the NOAEL of this study, since at the higher dose, statistically significant changes were observed in several kidney relevant parameter, among which NAG, which is an indicator or kidney damage.

A comparison of the NOAEL (320 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates in six human population groups of 0.010–0.095 mg TOS/kg bw per day at the mean and from 0.032 to 0.168 mg TOS/kg bw per day at the 95th percentile, resulted in margins of exposures (MOEs) above 1,905, indicating that there is no toxicological 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 allergenicity of maltogenic amylase produced with the genetically modified *Bacillus subtilis* strain NZYM-SM was assessed by comparing 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 sliding window of 80 amino acids as criterion, three matches were found. The matching allergens are: Asp o 21, an α-amylase produced by *Aspergillus oryzae*; Asp f 13, a serine protease produced by *Aspergillus fumigatus*; and Sch c 1, a glucoamylase produced by *Schizophyllum commune*.

α-Amylase from *A. oryzae* (Brisman and Belin, 1991; Brisman, 2002), serine protease from *A. fumigatus* (Kurup et al., 2002) and glucoamylase from *Schizophyllum commune* (Sander et al., 1998; Quirce et al., 2002) are all described as occupational respiratory allergens associated with baker's asthma. However, several studies have shown that adults with occupational asthma to a food enzyme (like α-amylase) can commonly ingest the corresponding enzyme without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). Taking into account the wide use of α-amylase, only a low number of case reports have been described in literature focussed on allergic reactions upon oral exposure to α-amylase in individuals respiratory sensitised to α-amylase (Baur and Czuppon, 1995; Kanny and Moneret-Vautrin, 1995; Moreno-Ancillo et al., 2004). In addition, such information is not reported for serine protease and glucoamylase. Therefore, it can be concluded that an allergic reaction upon oral ingestion of maltogenic amylase, produced by the genetically modified *B. subtilis* strain NZYM-SM, in individuals respiratory sensitised to α-amylase, serine protease produced by *A. fumigatus* or glucoamylase produced by *Schizophyllum commune* cannot be excluded, but the likelihood is considered to be low. Moreover, no information is available on oral sensitisation or elicitation reactions of this maltogenic amylase.

Bindslev-Jensen et al. (2006) investigated the cross reactivity of 19 different commercial enzymes used in the food industry in allergic patients (400 patients allergic to inhalation allergens, food allergens, allergens of bee or wasp or drugs). A maltogenic amylase from a *B. subtilis* species only gave a positive skin prick test in two allergic patients. Nevertheless, it was further tested by ingestion (DBPCFC) and was found to be negative to both active and placebo.

According to the information provided, substances or products that may cause allergies ([redacted]) or intolerances (Regulation EU 1169/2011)[7] are used as raw materials in the media fed to the microorganisms. However, these substances will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme. In addition, the microbial biomass and fermentation solids will be removed. Therefore, potentially allergenic residues of these foods employed as protein sources are not expected to be present.

Taken together, the Panel considers that there are no indications for allergic reactions by dietary exposure to the food enzyme maltogenic amylase produced with the genetically modified *B. subtilis* strain NZYM-SM.

## Conclusions

Based on the genetic modifications performed, the manufacturing process, the compositional and biochemical data provided, the dietary exposure assessment and the findings in the toxicological studies and the allergenicity assessment, the Panel concluded that the food enzyme maltogenic amylase from *Bacillus subtilis* strain NZYM-SM does not give rise to safety concerns under the intended conditions of use.

## Recommendations

## Documentation provided to EFSA

1) Dossier 'Maltogenic amylase produced by a genetically modified strain of *Bacillus subtilis* (strain NZYM-SM)'. February 2015. Submitted by Novozymes A/S.
2) Summary report on genotoxicity and subchronic toxicity study related to maltogenic alpha-amylase produced with a strain of *Bacillus subtilis* (strain NZYM-SM). March 2016. Delivered by FoBiG GmbH, Freiburg (Germany).
3) Additional information on 'Food enzyme carry/over in glucose syrups'. February 2017. Provided by the Association of Manufacturers and Formulators of Enzyme Products.
4) Additional information was received from Novozymes A/S in November 2017.
5) Additional information was received from Novozymes A/S in January 2018.

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[7] REGULATION (EU) No 1169/2011 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

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## Abbreviations

| | |
|---|---|
| bw | body weight |
| CAS | Chemical Abstracts Service |
| CFU | colony forming units |
| DBPCFC | double blind, placebo controlled food challenge |
| [redacted] | [redacted] |

| | |
|---|---|
| EC | Enzyme Commission |
| EINECS | European Inventory of Existing Commercial Chemical Substances |
| FAO | Food and Agricultural Organization |
| GLP | Good Laboratory Practice |
| GMO | genetically modified organism |
| GMP | Good Manufacturing Practice |
| IUBMB | International Union of Biochemistry and Molecular Biology |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| LOD | limit of detection |
| LOQ | limit of quantification |
| MOE | margin of exposure |
| NAG | *N*-acetyl-β-D-glucosaminidase |
| NOAEL | no observed adverse effect level |
| OECD | Organisation for Economic Cooperation and Development |
| PCR | polymerase chain reaction |
| PHA | phytohaemagglutinin |
| SDS–PAGE | sodium dodecyl sulfate–poly acrylamide gel electrophoresis |
| TOS | Total Organic Solids |
| WHO | World Health Organization |

# 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 on 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 | 15.0 |
| A.01.03 | Grain milling products (unspecified) | 1 | 1 | 15.0 |
| A.01.03.001 | Wheat milling products (unspecified) | 1 | 1 | 15.0 |
| A.01.03.001.001 | Wheat flour, brown | 1 | 1 | 15.0 |
| A.01.03.001.002 | Wheat flour, Durum | 1 | 1 | 15.0 |
| A.01.03.001.003 | Wheat flour, white | 1 | 1 | 15.0 |
| A.01.03.001.004 | Wheat flour, wholemeal | 1 | 1 | 15.0 |
| A.01.03.001.005 | Graham flour | 1 | 1 | 15.0 |
| A.01.03.001.006 | Wheat flour, gluten free | 1 | 1 | 15.0 |
| A.01.03.001.014 | Wheat starch | 1.2 | 1 | 15.0 |
| A.01.03.002 | Rye milling products (unspecified) | 1 | 1 | 15.0 |
| A.01.03.002.001 | Rye flour, gluten free | 1 | 1 | 15.0 |
| A.01.03.002.002 | Rye flour, light | 1 | 1 | 15.0 |
| A.01.03.002.003 | Rye flour, medium | 1 | 1 | 15.0 |
| A.01.03.002.004 | Rye flour, wholemeal | 1 | 1 | 15.0 |
| A.01.03.003 | Buckwheat milling products (unspecified) | 1 | 1 | 15.0 |
| A.01.03.003.001 | Buckwheat flour | 1 | 1 | 15.0 |
| A.01.03.004 | Corn milling products (unspecified) | 1 | 1 | 15.0 |
| A.01.03.004.001 | Corn flour | 1 | 1 | 15.0 |
| A.01.03.004.003 | Corn starch | 1.3 | 1 | 15.0 |
| A.01.03.005 | Oat milling products (unspecified) | 1 | 1 | 15.0 |
| A.01.03.005.002 | Oat flour | 1 | 1 | 15.0 |
| A.01.03.005.004 | Oat starch | 1.2 | 1 | 15.0 |
| A.01.03.006 | Rice milling products (unspecified) | 1 | 1 | 15.0 |
| A.01.03.006.001 | Rice flour | 1 | 1 | 15.0 |
| A.01.03.006.002 | Rice flour white | 1 | 1 | 15.0 |
| A.01.03.006.003 | Rice flour, instant | 1 | 1 | 15.0 |
| A.01.03.006.004 | Rice starch | 1.2 | 1 | 15.0 |
| A.01.03.007 | Spelt milling products | 1 | 1 | 15.0 |
| A.01.03.008 | Other milling products (unspecified) | 1 | 1 | 15.0 |
| A.01.03.008.001 | Amaranth flour | 1 | 1 | 15.0 |
| A.01.03.008.002 | Barley flour | 1 | 1 | 15.0 |
| A.01.03.008.003 | Chapatti flour | 1 | 1 | 15.0 |
| A.01.03.008.004 | Flour mix, wheat/rye/barley/oats | 1 | 1 | 15.0 |
| A.01.03.008.005 | Millet flour | 1 | 1 | 15.0 |
| A.01.03.008.007 | Sorghum flour | 1 | 1 | 15.0 |
| A.01.04 | Bread and rolls (unspecified) | 1 | 0.7 | 15.0 |
| A.01.04.001 | Wheat bread and rolls | 1 | 0.7 | 15.0 |
| A.01.04.002 | Rye bread and rolls | 1 | 0.7 | 15.0 |
| A.01.04.003 | Mixed wheat and rye bread and rolls | 1 | 0.7 | 15.0 |
| A.01.04.004 | Multigrain bread and rolls | 1 | 0.7 | 15.0 |
| A.01.04.005 | Unleavened bread, crisp bread and rusk (unspecified) | 1 | 0.8 | 15.0 |
| A.01.04.005.001 | Crisp bread, rye wholemeal | 1 | 0.9 | 15.0 |

| FoodEx code | FoodEx category | Conversion factor from FoodEx food group to raw material[a] | Recipe fraction[b] | mg TOS/ kg flour |
|---|---|---|---|---|
| A.01.04.005.002 | Crisp bread, rye, light | 1 | 0.9 | 15.0 |
| A.01.04.005.003 | Crisp bread, wheat, wholemeal | 1 | 0.9 | 15.0 |
| A.01.04.005.004 | Crisp bread, wheat, light | 1 | 0.9 | 15.0 |
| A.01.04.005.005 | Rusk, light | 1 | 0.9 | 15.0 |
| A.01.04.005.006 | Rusk, wholemeal | 1 | 0.9 | 15.0 |
| A.01.04.005.007 | Pita bread | 1 | 0.7 | 15.0 |
| A.01.04.005.008 | Matzo | 1 | 0.9 | 15.0 |
| A.01.04.005.009 | Tortilla | 1 | 0.7 | 15.0 |
| A.01.04.006 | Other bread | 1 | 0.7 | 15.0 |
| A.01.04.007 | Bread products | 1 | 0.7 | 15.0 |
| A.01.07 | Fine bakery wares (unspecified) | 1 | 0.5 | 15.0 |
| A.01.07.001 | Pastries and cakes (unspecified) | 1 | 0.5 | 15.0 |
| A.01.07.001.001 | Beignets | 1 | 0.15 | 15.0 |
| A.01.07.001.002 | Buns | 1 | 0.7 | 15.0 |
| A.01.07.001.003 | Cake from batter | 1 | 0.25 | 15.0 |
| A.01.07.001.004 | Cheese cream cake | 1 | 0.24 | 15.0 |
| A.01.07.001.005 | Cheese cream sponge cake | 1 | 0.24 | 15.0 |
| A.01.07.001.006 | Chocolate cake | 1 | 0.24 | 15.0 |
| A.01.07.001.007 | Chocolate cake with fruits | 1 | 0.24 | 15.0 |
| A.01.07.001.008 | Cream cake | 1 | 0.24 | 15.0 |
| A.01.07.001.009 | Cream cheese cake | 1 | 0.24 | 15.0 |
| A.01.07.001.010 | Cream custard cake | 1 | 0.24 | 15.0 |
| A.01.07.001.011 | Cream custard sponge cake | 1 | 0.24 | 15.0 |
| A.01.07.001.012 | Croissant | 1 | 0.5 | 15.0 |
| A.01.07.001.013 | Croissant, filled with chocolate | 1 | 0.5 | 15.0 |
| A.01.07.001.014 | Croissant, filled with cream | 1 | 0.5 | 15.0 |
| A.01.07.001.015 | Croissant, filled with jam | 1 | 0.5 | 15.0 |
| A.01.07.001.016 | Croquembouche | 1 | 0.15 | 15.0 |
| A.01.07.001.017 | Doughnuts | 1 | 0.24 | 15.0 |
| A.01.07.001.018 | Clair | 1 | 0.15 | 15.0 |
| A.01.07.001.019 | Flan | 1 | 0.5 | 15.0 |
| A.01.07.001.020 | Fruit cake | 1 | 0.6 | 15.0 |
| A.01.07.001.021 | Fruit pie | 1 | 0.15 | 15.0 |
| A.01.07.001.022 | Cheese pie | 1 | 0.15 | 15.0 |
| A.01.07.001.023 | Fruit tart | 1 | 0.15 | 15.0 |
| A.01.07.001.024 | Gingerbread | 1 | 0.6 | 15.0 |
| A.01.07.001.025 | Gougere | 1 | 0.15 | 15.0 |
| A.01.07.001.026 | Kringles | 1 | 0.25 | 15.0 |
| A.01.07.001.027 | Nut cream cake | 1 | 0.24 | 15.0 |
| A.01.07.001.028 | Pancakes | 1 | 0.25 | 15.0 |
| A.01.07.001.029 | Profiterole | 1 | 0.15 | 15.0 |
| A.01.07.001.030 | Pyramid cake | 1 | 0.25 | 15.0 |
| A.01.07.001.031 | Rhubarb flan | 1 | 0.15 | 15.0 |
| A.01.07.001.032 | Scone | 1 | 0.5 | 15.0 |
| A.01.07.001.033 | Sponge dough | 1 | 0.25 | 15.0 |
| A.01.07.001.034 | Sponge cake | 1 | 0.25 | 15.0 |
| A.01.07.001.035 | Sponge cake roll | 1 | 0.25 | 15.0 |

| FoodEx code | FoodEx category | Conversion factor from FoodEx food group to raw material[a] | Recipe fraction[b] | mg TOS/ kg flour |
|---|---|---|---|---|
| A.01.07.001.036 | Muffins | 1 | 0.25 | 15.0 |
| A.01.07.001.037 | Waffles | 1 | 0.25 | 15.0 |
| A.01.07.001.038 | Apple strudel | 1 | 0.15 | 15.0 |
| A.01.07.001.039 | Cream-cheese strudel | 1 | 0.24 | 15.0 |
| A.01.07.001.040 | Cheese pastry goods from puff pastry | 1 | 0.15 | 15.0 |
| A.01.07.001.041 | Croissant from puff pastry | 1 | 0.6 | 15.0 |
| A.01.07.001.042 | Brioche | 1 | 0.5 | 15.0 |
| A.01.07.001.044 | Lebkuchen | 1 | 0.6 | 15.0 |
| A.01.07.001.045 | Dumpling | 1 | 0.5 | 15.0 |
| A.01.07.001.046 | Cake marbled, with chocolate | 1 | 0.5 | 15.0 |
| A.01.07.001.047 | Marzipan pie | 1 | 0.25 | 15.0 |
| A.01.07.001.048 | Baklava | 1 | 0.15 | 15.0 |
| A.01.07.002 | Biscuits (cookies) | 1 | 0.9 | 15.0 |
| A.01.07.002.001 | Biscuits, sweet, plain | 1 | 0.9 | 15.0 |
| A.01.07.002.002 | Biscuits, chocolate filling | 1 | 0.81 | 15.0 |
| A.01.07.002.003 | Biscuits, cream filling | 1 | 0.81 | 15.0 |
| A.01.07.002.004 | Biscuits, fruit filling | 1 | 0.81 | 15.0 |
| A.01.07.002.005 | Biscuits, vanilla filling | 1 | 0.81 | 15.0 |
| A.01.07.002.006 | Butter biscuits | 1 | 0.81 | 15.0 |
| A.01.07.002.007 | Biscuit, iced | 1 | 0.81 | 15.0 |
| A.01.07.002.008 | Speculaas | 1 | 0.9 | 15.0 |
| A.01.07.002.009 | Biscuits, sweet, wheat wholemeal | 1 | 0.9 | 15.0 |
| A.01.07.002.010 | Biscuits, oat meal | 1 | 0.9 | 15.0 |
| A.01.07.002.011 | Biscuits, spelt meal | 1 | 0.9 | 15.0 |
| A.01.07.002.012 | Biscuits, salty | 1 | 0.9 | 15.0 |
| A.01.07.002.013 | Biscuits, salty, with cheese | 1 | 0.81 | 15.0 |
| A.01.07.002.014 | Sticks, salty | 1 | 0.81 | 15.0 |
| A.17.03.003 | Biscuits, rusks and cookies for children | 1 | 0.9 | 15.0 |
| A.18.04.001 | Find bakery products for diabetics | 1 | 0.5 | 15.0 |
| A.19.01.001 | Sandwich and sandwich-like meal | 1 | 0.32 | 15.0 |
| A.19.01.002 | Pizza and pizza-like pies | 1 | 0.3 | 15.0 |

TOS: Total Organic Solids.
(a): Available at see 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 the 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 provided in this appendix is shown in an excel file (downloadable http://onlinelibrary.wiley.com/wol1/doi/10.2903/j.efsa.2018.5171/suppinfo).

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.