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Safety of the food enzyme glucoamylase from a genetically modified *Aspergillus niger* (strain NZYM-BF)

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Abstract

The food enzyme glucoamylase (glucan 1,4-α-glucosidase; EC 3.2.1.3) is produced with the genetically modified strain of *Aspergillus niger* by Novozymes A/S. The genetic modifications do not give rise to safety concerns. The food enzyme is free from viable cells of the production organism and recombinant DNA. This glucoamylase is intended to be used in brewing processes and in starch processing for glucose syrups production. Residual amounts of total organic solids (TOS) are removed by the purification steps applied during the production of glucose syrups, consequently dietary exposure was not calculated. For brewing processes, based on the proposed maximum use levels, dietary exposure to the food enzyme–TOS was estimated to be below 3.627 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not raise a safety concern. The systemic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rodents. The Panel identified a no-observed-adverse-effect level (NOAEL) at the highest dose of 1,360 mg TOS/kg bw per day. Similarity of the amino acid sequence to those of known allergens was searched and one match was found. The Panel considered 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 to occur is considered to be low. Based on the data provided, the removal of TOS during the production of glucose syrups and the derived margin of exposure for brewing processes, the Panel concluded that this food enzyme does not raise safety concerns under the intended conditions of use.

Keywords: food enzyme, glucoamylase, amyloglucosidase, glucan 1, 4-α-glucosidase, EC 3.2.1.3, *Aspergillus niger*, 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 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 entered 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 an approval via a Union list.

The 'Guidance on submission of a dossier on a food enzyme for 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 companies 'DSM Food Specialities B.V.' and 'Novozymes A/S' for the authorisation of the food enzymes Endo-1,4-beta-xylanase from a genetically modified strain of *Aspergillus niger* (strain XYL), Alpha-amylase from a genetically modified strain of *Aspergillus niger* (strain NZYM-MC) and Glucoamylase from a genetically modified strain of *Aspergillus niger* (strain NZYM-BF).

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 Endo-1,4-beta-xylanase modified strain of *Aspergillus niger* (strain XYL), Alpha-amylase from a genetically modified strain of *Aspergillus niger* (strain NZYM-MC) and

[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 1493/199, 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.

Glucoamylase from a genetically modified strain of *Aspergillus niger* (strain NZYM-BF) 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 request to carry out the safety assessment of the food enzyme Glucoamylase from a genetically modified strain of *A. niger* (strain NZYM-BF).

1.3. Information on existing authorisations and evaluations

The applicant indicates that the food enzyme object of the present dossier has not been evaluated by authorities in the EU. The applicant also reports that the Brazilian, Canadian, Chinese, Danish, French, Mexican, Russian and South Korean authorities have evaluated and authorised the use of glucoamylases and/or glucoamylases from genetically modified strains of *A. niger.*[4]

2. Data and methodologies

2.1. Data

The applicant submitted a dossier supporting the application for authorisation of the food enzyme glucoamylase from a genetically modified strain of *Aspergillus niger* (strain NZYM-BF).

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 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

IUBMB nomenclature:	Glucan 1,4-α-glucosidase
Systematic name:	4-α-D-glucan glucohydrolase
Synonyms:	Glucoamylase, amyloglucosidase
IUBMB No:	EC 3.2.1.3
CAS No:	9032-08-0
EINECS No:	232-877-2.

Glucoamylase catalyses the hydrolysis of 1,4-α linkages in the starch polysaccharides, amylose and amylopectin, and their hydrolysis' products (dextrins) releasing free D-glucose. It also catalyses the hydrolysis of 1,6-α linkages in amylopectin, but at a slower rate.[5] It is intended to be used in starch processing for glucose syrups production and brewing processes.

3.1. Source of the food enzyme

The glucoamylase is produced with a genetically modified filamentous fungus *Aspergillus niger*. The technical dossier contains all necessary information on the recipient microorganism, the donor organism and the genetic modification process.

The production strain *A. niger* NZYM-MC is deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GMbH (DSMZ, Germany) with deposit number [redacted].

[4] Technical dossier: p. 27.
[5] Technical dossier: p. 40.

3.1.1. Characteristics of the parental and recipient microorganisms

The parental strain is *A. niger* [redacted]. The first intermediate strain [redacted] is deposited at the German Collection of Microorganisms and Cell Cultures with accession number [redacted]. It was derived from the parental strain by [redacted]. Strain [redacted] was identified as *A. niger* by [redacted].

Recipient strain *A. niger* [redacted].

An isolate with improved glucoamylase production was identified after [redacted].

During the development of the recipient strain [redacted].

3.1.2. Characteristics of the introduced sequences

The gene encoding the [redacted].

3.1.3. Description of the genetic modification process

The purpose of genetic modification was to enable the production strain to synthesise glucoamylase [redacted].

The recipient strain [redacted].

3.1.4. Safety aspects of the genetic modification

The production strain *A. niger* NZYM-BF differs from the recipient strain [redacted] only in its capacity to produce the glucoamylase enzyme from [redacted] The presence and the location of the *enzyme* gene were confirmed by Southern analysis. The phenotypic stability of the *A. niger* NZYM-BF strain was confirmed by its capacity to produce a constant level of the enzyme glucoamylase measured in relation to the TOS in three independent batches of the food enzyme and its genetic stability was demonstrated by Southern analysis with DNA isolated from end-of-production samples from three different batches.[6]

The absence of the antibiotic resistance genes used during the genetic modification was confirmed by Southern analysis of the production strain NZYM-BF with probes specific to [redacted] resistance genes. The absence of the [redacted] was also confirmed by Southern analysis.[7]

No issues of concern arising from the genetic modifications were identified by the Panel.

3.2. Production of the food enzyme

The food enzyme is manufactured according to Food Hygiene Regulation (EC) No 852/2004[8], with food safety procedures based on Hazard Analysis and Critical Control Points (HACCP), and in accordance with current Good Manufacturing Practice (GMP).

The production strain is grown as a pure culture using a typical industrial medium in a submerged, fed-batch fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration leaving a supernatant containing the food enzyme. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in which enzyme protein is retained while most of the low molecular weight material passes the filtration membrane and is discarded. The applicant provided information on the identity of the substances used to control the fermentation and in the subsequent downstream processing of the food enzyme.

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

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The glucoamylase is a single polypeptide chain of 595 amino acids. The molecular mass, based on the amino acid sequence, was calculated to be 65.5 kDa.[9] The homogeneity of the food enzyme was investigated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. The gels presented for the four food enzyme batches are comparable, showing one major protein band occurring in the expected region.[10] The food enzyme was tested for lipase and protease activities and no relevant activities were detected.[11] No other enzymatic side activities were reported.

The in-house determination of glucoamylase activity is based on hydrolysis of maltose and is expressed in Amyloglucosidase Units/g (AGU/g), (reaction conditions: pH = 4.3, T = 37°C, incubation time 6 min).[12] The enzyme activity is determined based on the released glucose and calculated relative to an enzyme standard.[13]

The food enzyme has been characterised with regard to its temperature and pH profiles. It has a temperature optimum around 70°C (pH = 5.0) and a pH optimum around 4.0 (T = 37°C). Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures. Under the conditions (pH = 5.0) of the applied temperature stability assay, the glucan 1,4-α-glucosidase activity decreased rapidly above 60°C showing no residual activity above 80°C.[14]

[6] Annex D2.
[7] Annex D1.
[8] 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, 321 pp.
[9] Technical dossier: p. 34 and Annex 1.
[10] Technical dossier: p. 36.
[11] Technical dossier: p. 44 and Annex 3.
[12] Annex 3.01.
[13] Technical dossier: p. 41.
[14] Technical dossier: p. 42–43 and Annex 9.

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme have been 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 commercial enzyme batches was 11.9% (range 11.4–12.2%). The average enzyme activity/TOS ratio of the three batches for commercialisation is 3.16 AGU/mg TOS.

Table 1: Compositional data of the food enzyme[(d)]

Parameter	Unit	Batch			
		1	2	3	4[(a)]
Glucoamylase activity	AGU/g batch[(b)]	422	361	343	187
Protein	%	9.3	9.6	8.9	8.9
Ash	%	0.3	0.3	0.6	0.3
Water	%	87.7	87.5	88.0	86.7
Total organic solids (TOS)[(c)]	%	12.0	12.2	11.4	13.0
Glucoamylase activity/mg TOS	AGU/mg TOS	3.52	2.96	3.01	1.44

(a): Batch used for the toxicological tests.
(b): AGU: AmyloGlucosidase Units (see Section 3.3.1).
(c): TOS calculated as 100% – % water – % ash.
(d): Technical dossier: p. 35, Annex 2 and Additional information received in September 2017.

3.3.3. Purity

The food enzyme complies with the specification for lead (not more than 5 mg/kg)[15] as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006). In addition, the applicant provided that the levels of arsenic, cadmium and mercury were below the limits of detection of the employed methods.[16]

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 *E. coli* and *Salmonella* species are absent in 25 g of sample and total coliforms are not more than 30 colony forming units (CFU) per gram.[17] No antimicrobial activity was detected in four batches of food enzyme (FAO/WHO 2006).

Mycotoxins, particularly ochratoxin A and fumonisins, are produced by many strains of *A. niger* (Blumenthal, 2004; Frisvad et al., 2007, 2011; EFSA BIOHAZ Panel, 2018). [redacted] the strain unable to produce ochratoxin A and fumonisins. This was confirmed by analysis of the four batches of food enzyme in which the levels of these mycotoxins were below the limits of detection.[18]

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

3.3.4. Viable cells and DNA of the production strain

The absence of the production strain in the food enzyme was demonstrated in three independent batches analysed in triplicate. [redacted]

A test for recombinant DNA in the food enzyme was made by polymerase chain reaction (PCR) analysis of three batches in triplicate. [redacted]

[15] Technical dossier: p. 37 and Annex 2.
[16] LOD: Pb: 5 mg/kg; As: 0.1 mg/kg; Cd: 0.05 and Hg: 0.03 mg/kg
[17] Technical dossier: p. 39 and Annex 3
[18] Technical dossier: p. 37 and Annex 3.

3.4. Toxicological data

The food enzyme batch 4 (Table 1) used for toxicological assays has been concentrated by evaporation and not by ultrafiltration as the commercial batches.[19] This resulted in higher TOS content relative to activity compared to the commercial batches, and thus, batch 4 is considered cruder and suitable for the toxicological testing.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test[20]

In order to investigate the potential of food enzyme to induce gene mutations, a bacterial reverse mutation test (Ames test) was performed according to OECD Test Guideline 471 (OECD, 1997) and following Good Laboratory Practice (GLP) in four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *E. coli* WP2 *urv*A (pKM101), in the presence or absence of metabolic activation by applying the 'treat and plate assay'. Two separate experiments were carried out using six different concentrations of the food enzyme (156, 313, 625, 1,250, 2,500 and 5,000 μg dry matter/mL, corresponding to ca. 150, 300, 610, 1,200, 2,400 and 4,900 μg TOS/mL) and appropriate positive and negative controls.

No reduction in the growth of the background lawn was observed at any concentration. However, in experiment 1 with TA100 in the absence of S9, increases, not concentration related and not reproduced in the experiment 2 were observed. Additionally, in the experiment 2, a clear growth stimulating effect of the food enzyme on strain TA100 in the absence of S9 mix was seen. These effects were not considered relevant. No significant increase in the revertant colony numbers was observed at any concentration of the food enzyme. 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.

3.4.1.2. *In vitro* mammalian cell micronucleus test[21]

The *in vitro* micronucleus assay was carried out according to OECD Test Guideline 487 (OECD, 2010) and following GLP. Lymphocyte cultures were treated with the food enzyme at three concentrations (3,000, 4,000 and 5,000 μg food enzyme/mL, corresponding to 390, 520 and 650 μg TOS/mL) in a single experiment for 3 + 21 h in the presence and absence of S9-mix (short treatment) and for 24 + 24 h absence of S9-mix (continuous treatment). Appropriate negative and positive controls were used. No cytotoxicity was observed up to 5,000 μg/mL food enzyme. A statistically significant increase of micronucleated binucleated (MNBN) cells, reproduced in both replicate cultures, was observed at 650 μg TOS/mL for 24 + 24 h treatment without S9 mix, but the values of MNBN cells were clearly within the 95th percentile of the historical negative control range. No increase in the frequency of micronuclei was observed at all the other concentrations analysed. The Panel concluded that the food enzyme did not induce micronuclei in cultured human peripheral blood lymphocytes when tested up to 5,000 μg food enzyme/mL, corresponding to 650 μg TOS/mL under the experimental conditions employed.

3.4.2. Repeated dose 90-day oral toxicity study in rodents[22]

A repeated dose 90-day oral toxicity study was performed according to OECD Test Guideline 408 (OECD, 1998) and following GLP. Four groups of 10 male and 10 female Sprague–Dawley Crl:CD(SD) rats received the food enzyme by gavage corresponding to 136, 449 or 1,360 mg TOS/kg body weight (bw) per day. Controls received the vehicle (water).

No mortality was observed.

Motor activity examination showed that high-dose males had slightly but statistically significantly decreased total rearing and cage floor activity scores compared to the controls. The total scores for these were within historical control ranges, but it was also noted that the low beam break score for the control animals was higher than would be expected from the historical range. Statistically significant reductions in scores were recorded for cage floor activity in males during the second (high dose) and seventh 6-minute intervals (mid- and high dose) and for females during the second (at low

[19] Technical dossier: p. 35.
[20] Annex 7.01
[21] Annex 7.02.
[22] Annex 7.03.

dose), seventh and tenth (both at high dose) 6-minute intervals, but not at the other time points. Because of the isolated nature of these findings, and that all reduced values were within the range of the historical control data, they were considered incidental and reflecting normal variation.

In haematological investigation, slightly but significantly extended blood coagulation times (activated partial thromboplastin times (APTT)) were seen in high-dose males and females. The differences to control values were small (< 2 sec). In females, the extended APTT were associated with a small reduction in prothrombin time, though all values were within the range of historical control data. In blood chemistry, a slight but statistically significant increase in plasma potassium concentration occurred in high-dose females. The majority of high-dose females had potassium concentrations above the range reported for the concurrent controls. These changes were considered of no toxicological relevance.

There was a statistically significant reduced absolute liver weight at all doses in females. As the differences from controls were minimal, and lacked dose relationship these differences were considered to represent normal biological variation.

Overall, the Panel noted that the differences to controls in haematological and clinical chemistry parameters were without apparent dose–response relationship and the values were within historical control ranges from the laboratory. Regarding, the recorded difference in liver weights, the Panel noted that the concurrent control value was in the high while in the treated groups it was in the low end of historical control range. Additionally, no treatment-related microscopical changes were seen in the livers from treated groups. Accordingly, the Panel considered that the no-observed-adverse-effect level (NOAEL) in this study was 1,360 mg TOS/kg bw per day, the highest dose tested.

3.4.3. 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 this glucoamylase produced with the genetically modified *A. niger* strain NZYM-BF was assessed by comparing its amino acid sequence with those of known allergens according to the scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms (EFSA GMO Panel, 2010). Using higher than 35% identity in a window of 80 amino acids as the criterion, one match was found. The matching allergen was Sch c 1, a glucoamylase from *Schizophyllum commune*, an enzyme described as an occupational respiratory allergen associated with baker's asthma (Quirce et al., 2002; Toyotome et al., 2014).

No information is available on oral sensitisation or elicitation reactions of this glucoamylase. Several studies have shown that adults with occupational asthma may be able to ingest respiratory allergens without acquiring clinical symptoms of food allergy (Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). In addition, no allergic reactions upon dietary exposure to any glucoamylase have been reported in the literature. Therefore, it can be concluded that the likelihood of an allergic reaction upon oral ingestion of this glucoamylase, produced with the genetically modified *A. niger* strain NZYM-BF, in individuals respiratory sensitised to glucoamylase, cannot be excluded, but the likelihood of such a reaction to occur is considered to be low.

According to the information provided, substances or products that may cause allergies [redacted] or intolerances (Regulation EU 1169/2011)[23] are used as raw materials in the growth medium of the production organism. However, during the fermentation process, these products will be degraded and utilised by the fungus for cell growth, cell maintenance and production of enzyme protein. In addition, the fungal biomass and fermentation solids will be removed. Considering the fermentation process and downstream processing, the Panel considered that potentially allergenic residues of these foods employed as protein sources are not expected to be present.

Quantifying the risk for allergenicity is not possible in view of the individual susceptibility to food allergens. Allergenicity can be ruled out only if the proteins are fully removed. In the starch processing for the production of glucose syrups, experimental data showed a significant removal (> 99%) of protein. However, traces of protein could be present in glucose syrup.

[23] 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.

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

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in starch processing for glucose syrups production and brewing processes at the recommended use levels summarised in Table 2.

Table 2: Intended uses and recommended use levels of the food enzyme as provided by the applicant[(b),(c)]

Food manufacturing process[(a)]	Raw material	Recommended dosage of the food enzyme
Starch processing for glucose syrups production	Starch dry matter	237 mg TOS/kg starch dry matter (up to 750 AGU/kg of starch dry matter)
Brewing processes (beer)	Malted barley	791 mg TOS/kg malted barley (up to 2,500 AGU/kg of malted barley)

TOS: Total Organic Solids; AGU: AmyloGlucosidase Units.
(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): The original intended uses proposed by the applicant were: 'Baking processes' and 'Cereal-based processes'. In the course of the evaluation, the applicant informed EFSA about withdrawal of the originally applied intended use in 'Baking processes' and 'Cereal-based processes'.
(c): Technical dossier: p. 60 and Additional information received in September 2017.

In starch processing for glucose syrups production, glucoamylase is added during the saccharification step.

In brewing processes, the glucoamylase is added during the mashing step in order to obtain a more uniform and predictable production process and yield, including the possibility to control the desired level of fermentable sugars.

Experimental data have been provided on the removal (> 99%) of protein in the course of starch processing for the production of glucose syrups (Documentation provided to EFSA No 4). 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.

The food enzyme remains in the beer. Based on data provided on thermostability (see Section 3.3.1), it is anticipated that the glucoamylase is inactivated during brewing processes.

3.5.2. Dietary exposure estimation

As residual amounts of TOS are removed by the purification steps applied during the production of glucose syrups (by > 99%), foods/ingredients derived through starch processing, i.e. glucose syrups were excluded from the estimation.

For the brewing processes, chronic exposure was calculated using the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016). The assessment involved selection of relevant food categories from the EFSA Comprehensive European Food Consumption Database[24] and application of process and technical conversion factors (Annex B in EFSA CEF Panel, 2016).

Chronic exposure was calculated by combining the maximum recommended use level provided by the applicant (see Table 2) with the relevant FoodEx categories (Annex B in EFSA CEF Panel, 2016), based on individual consumption data. Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period and normalised for bodyweight. This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only one day per subject were excluded and high-level

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

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

Table 3 provides an overview of the derived exposure estimates across all surveys. Detailed average and 95th percentile exposure to the food enzyme–TOS per age class, country and survey, as well as contribution from each FoodEx category to the total dietary exposure are reported in Appendix A – Tables 1 and 2. For the present assessment, food consumption data were available from 35 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B).

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

Population group	Estimated exposure (mg/kg body weight per day)					
	Infants	**Toddlers**	**Children**	**Adolescents**	**Adults**	**The elderly**
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0 (10)	0–0.010 (14)	0–0.019 (19)	0–0.154 (18)	0.061–0.805 (19)	0.016–0.397 (18)
Min–max 95th percentile (number of surveys)	0 (8)	0 (12)	0 (19)	0–0.957 (17)	0.448–3.627 (19)	0.100–1.658 (18)

3.5.3. Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties have been considered and are summarised in Table 4.

Table 4: Qualitative evaluation of the influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction of impact
Model input data	
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/–
Use of data from food consumption 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 based on the description of the food process provided by the applicant	+
Use of recipe fractions in disaggregation FoodEx categories	+/–
Use of technical factors in the exposure model	+/–

+: uncertainty with potential to cause overestimation of exposure; –: uncertainty with potential to cause underestimation of exposure.

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

3.6. Margin of exposure

A comparison of the NOAEL (1,360 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates of 0–0.805 mg TOS/kg bw per day at the mean and from 0 to 3.627 mg TOS/kg bw per day at 95th percentile, resulted in margin of exposure (MOE) above 375.

4. Conclusions

Based on the data provided, the removal of TOS during the production of glucose syrups and the margin of exposure calculated when used in brewing processes, the Panel concludes that the food enzyme glucoamylase produced with the genetically modified *A. niger* strain NZYM-BF does not give rise to safety concerns under the intended conditions of use.

The CEP Panel considers the food enzyme free from viable cells of the production organism and recombinant DNA.

Documentation provided to EFSA

1) Dossier 'Glucoamylase produced by a genetically modified strain of *Aspergillus niger* (strain NZYM-BF)'. August 2013. Submitted by Novozymes A/S.
2) Additional information submitted in March 2015 by the applicant.
3) Additional information submitted in July 2015 by the applicant.
4) Additional information on 'Food enzyme carry/over in glucose syrups.' February 2017. Provided by the Association of Manufacturers and Formulators of Enzyme Products.
5) Additional information submitted in September 2017 by the applicant.
6) Additional information submitted in March 2018 by the applicant.
7) Summary report on genotoxicity, subchronic toxicity study and allergenicity for glucoamylase produced with a genetically modified strain of *Aspergillus niger* (strain NZYM-BF) by Novozymes A/S. Delivered by FoBiG GmbH (Freiburg, Germany) on 5 August 2014.

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Abbreviations

AGU	AmyloGlucosidase Units
APTT	activated partial thromboplastin times
bp	base pair
bw	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes, Processing Aids
CFU	colony forming units
EC	Enzyme Commission
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organisation
[redacted]	[redacted]
GLP	Good Laboratory Practice
GM	genetically modified
GMO	Panel EFSA Scientific Panel on Genetically Modified Organisms
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points
ITS	internal transcribed spacer
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
MNBN	micronucleated binucleated
MOE	margin of Exposure
NOAEL	no-observed-adverse-effect level
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
SDS-PAGE	sodium dodecyl sulfate-poly acrylamide gel electrophoresis
TOS	total organic solids
WHO	World Health Organization

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

Information provided in this appendix is shown in an excel file (downloadable here).

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 FoodEx categories to the food enzyme–TOS dietary exposure.

Appendix B – Population groups considered for the exposure assessment

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

(a): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011).