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Safety evaluation of the food enzyme α-amylase from a genetically modified *Aspergillus niger* (strain NZYM-MC)

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Abstract

The food enzyme alpha-amylase (4-α-D-glucan glucanohydrolase; EC 3.2.1.1) 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 α-amylase is intended to be used in starch processing for glucose syrups production, beverage alcohol (distilling) processes and baking processes. Residual amounts of total organic solids (TOS) are removed by distillation and by the purification steps applied during the production of glucose syrups, consequently dietary exposure was not calculated. For baking processes, based on the proposed maximum use levels, dietary exposure to the food enzyme–TOS was estimated to be up to 3.784 mg TOS/kg body weight 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 1,400 mg TOS/kg body weight (bw) per day. Similarity of the amino acid sequence to those of known allergens was searched and two matches were 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 baking processes, the Panel concluded that this food enzyme does not raise safety concerns under the intended conditions of use.

Keywords: food enzyme, α-amylase, 4-α-D-glucan glucanohydrolase, 1,4-α-D-glucan glucanohydrolase, EC 3.2.1.1, *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, pp. 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's request to carry out the safety assessment of the food enzyme Alpha-amylase from a genetically modified strain of *A. niger* (strain NZYM-MC).

1.3. Information on existing authorisations and evaluations

The applicant reports that the Danish authority has evaluated and authorised the use of α-amylase from a genetically modified strain of *A. niger* (strain NZYM-MC) in starch processing, distilled alcohol production and baking processes as well as set up the condition of use up to 200 FAU(F)/kg starch dry matter.[4]

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier supporting the application for authorisation of the food enzyme α-amylase from a genetically modified strain of *A. niger* (strain NZYM-MC).

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:	α-amylase
Systematic name:	4-α-D-glucan glucanohydrolase
Synonyms:	Endo-amylase, 1,4-α-D-glucan glucanohydrolase
IUBMB No:	EC 3.2.1.1
CAS No:	9000-90-2
EINECS No:	232-565-6

α-Amylase catalyses the hydrolysis of 1,4-α-glycosidic linkages in starch (amylose and amylopectin), resulting in the generation of oligosaccharides. It is intended to be used in starch processing for glucose syrups production, distilled alcohol production and baking processes.

3.1. Source of the food enzyme

The α-amylase 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].

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

[4] Technical dossier: p. 15.

the parental strain by [redacted]. Strain [redacted] was identified as *A. niger* by [redacted].

Recipient strain *A. niger* [redacted].

Additionally, the [redacted].

During the development of the recipient strain, [redacted].

3.1.2. Characteristics of the introduced sequences

The gene encoding [redacted].

3.1.3. Description of the genetic modification process

The purpose of genetic modification was to enable the production strain to synthesise α-amylase from [redacted].

3.1.4. Safety aspects of the genetic modification

The recipient strain *A. niger* [redacted] is unable to produce oxalic acid and fumonisin. It also does not produce detectable levels of ochratoxin A. The production strain *A. niger* NZYM-MC differs from the recipient strain [redacted] only in its capability to produce the α-amylase enzyme from [redacted] and [redacted]. The presence and the location of [redacted] gene copies were confirmed by Southern analysis. One copy of the [redacted] selectable marker is present at each insertion locus.

The absence of antibiotic resistance genes used during the genetic modification was confirmed by Southern blot. Absence of the [redacted] and the [redacted] were also confirmed. The phenotypic stability of the *A. niger* NZYM-MC strain was confirmed by its capacity to produce a constant level of the enzyme α-amylase 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.

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[5], 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 α-amylase is a single polypeptide chain of 583 amino acids. The molecular mass, based on the amino acid sequence, was calculated to be 63.5 kDa.[6] The homogeneity of the food enzyme was investigated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. The gels presented for four food enzyme batches are comparable, showing several protein bands including a band in the expected region.[7] The food enzyme was tested for lipase and protease activities, which were below the detection limits of the employed methods.[8] No other enzymatic side activities were reported.

The in-house determination of α-amylase activity is based on hydrolysis of the substrate 4,6-ethylidene(G7)-*p*-nitrophenyl(G1)-α-D-maltoheptaoside (ethylidene-G7*p*NP) which is cleaved to glucose and *p*-nitrophenol (reaction conditions: pH 7.2, temperature 37°C, reaction time 5 min). The enzymatic activity is quantified by measuring the formation of *p*-nitrophenol spectrophotometrically at 405 nm. The activity is quantified relative to an internal enzyme standard ('F') and expressed in Fungal α-amylase Units/g (FAU(F)/g).[9]

The food enzyme has been characterised with regard to its temperature and pH profiles. It is active at temperatures up to 80°C (with an optimum of 60°C at pH 5.5 and 30 min incubation) and within a pH range of 2–9 (with an optimum of pH 3 at 30°C and 30 min incubation).[10] Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures. Under the conditions (pH 5.5) of the applied temperature stability assay, α-amylase activity decreased above 60°C showing no residual activity above 80°C.[11]

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

[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, 321 pp.
[6] Technical dossier: p. 35 and Annex 1.
[7] Technical dossier: p. 37.
[8] Technical dossier: p. 43 and Additional information received in August 2017.
[9] Technical dossier: p. 41 and Annex 3.01.
[10] Technical dossier: p. 41 and Annex 9.
[11] Technical dossier: p. 42 and Annex 9.

(Table 1). The average total organic solids (TOS) content of the three commercial enzyme batches was 10.9% (range 10.1–11.8%). The average enzyme activity/TOS ratio of the three batches for commercialisation is 0.5 FAU(F)/mg TOS.

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

Parameter	Unit	Batch			
		1	2	3	4[(a)]
α-amylase activity	FAU(F)/g batch[(b)]	58.7	52.4	52.6	65.3
Protein	%	6.7	5.4	5.8	6.6
Ash	%	0.8	0.8	0.8	0.7
Water	%	87.4	88.5	89.1	86.0
Total organic solids (TOS)[(c)]	%	11.8	10.7	10.1	13.3
α-amylase activity/mg TOS	FAU(F)/mg TOS	0.50	0.49	0.52	0.49

(a): Batch used for the toxicological tests.
(b): FAU(F): Fungal α-amylase Units (relative to an internal standard 'F') (see Section 3.3.1).
(c): TOS calculated as 100% − % water − % ash.
(d): Technical data: p. 36 and p. 63 and Additional information received in October 2017.

3.3.3. Purity

The food enzyme complies with the specification for lead (not more than 5 mg/kg)[12] as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006). In addition, the levels of cadmium and mercury were below the limits of detection of the employed methodologies.[13] For arsenic, the average concentration determined in the commercial batches was 0.15 mg/kg.[14] Since under the conditions of use this would not add significantly to the total arsenic intake, the Panel considered this concentration was not of concern.

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 should not exceed 30 colony forming units (CFU) per gram.[15] 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.

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].

The absence of recombinant DNA in the enzyme product was demonstrated by polymerase chain reaction (PCR) analysis of three batches in triplicate. [redacted]

3.4. Toxicological data

A shown in Table 1 the food enzyme batch 4 is considered representative of the commercial batches, and thus suitable for toxicological testing.

[12] Technical dossier: p. 38 and Annex 2.04 and Additional information received in October 2017.
[13] LOD: Pb: 5 mg/kg; Cd: 0.05 mg/kg and Hg: 0.03 mg/kg.
[14] LOD: As: 0.1 mg/kg.
[15] Technical dossier: p. 40 and Annexes: 2.08, 2.09, 2.10, 2.11 and Additional information received in October 2017.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test[16]

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, 1997) and following Good Laboratory Practice (GLP) in four strains of *Salmonella* Typhimurium (TA1535, TA1537, TA98, and TA100) and *E. coli* WP2 *uvr*A (pKM101), in the presence or absence of metabolic activation applying a 'treat and plate' assay. Two separate 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/mL, corresponding to 148, 297, 594, 1,187, 2,375 and 4,750 μg TOS/mL) using appropriate positive controls and negative controls. No precipitation and growth inhibition were observed. However, growth stimulation, measured as increased viable count, 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.

3.4.1.2. *In vitro* mammalian cell micronucleus test[17]

The *in vitro* micronucleus assay was carried out according to the OECD Test Guideline 487 (OECD, 2010) and following GLP. Whole blood cultures were treated with the food enzyme at three concentrations (3,000, 4,000 and 5,000 μg food enzyme/mL, corresponding to 399, 532 and 665 μg TOS/mL) applying a short-term treatment (3 h followed by 21 h recovery) in the presence and absence of S9-mix, and a continuous treatment (24 h + 24 h recovery) in the absence of S9-mix. No cytotoxicity was observed up to the highest dose tested. A slight increase in one of the replicate cultures at concentration of 5,000 μg food enzyme/mL (corresponding to 665 μg TOS/mL) following short treatment in the presence of S9-mix (1.2% MNBN cells) was detected. However, this increase was marginal (95% reference range = 0.1–1.1%) and not observed in the replicate culture; therefore, it was not considered biologically relevant. No statistically significant increase in the frequency of micronuclei was observed in the treated cultures compared to the negative controls in all the other tested conditions. The Panel concluded that the food enzyme α-amylase did not induce micronuclei in cultured human peripheral blood lymphocytes when tested up to 665 μg TOS/mL under the test conditions employed in this study.

3.4.2. Repeated dose 90-day oral toxicity study in rodents[18]

A repeated dose 90-day oral toxicity study was performed according to OECD Test Guideline 408 (OECD, 1998) and following GLP. Groups of 10 male and 10 female Sprague–Dawley rats (strain HanTac:WH (GALAS)) received the food enzyme by gavage corresponding to 140, 462 or 1,400 mg TOS/kg body weight (bw) per day (referred to as low-dose, mid-dose and high-dose groups, respectively), with a dose volume of 10 mL/kg. A control group received tap water alone.

Mortality was observed on day 76 in one mid-dose male which on necropsy was diagnosed to be due to misdosing.

Among haematological parameters, the only statistically significant difference to controls was increase in mean cell volume in the low-dose females.

Statistically significant differences to controls males in clinical chemistry parameters included: an increased protein level in the low-dose group, lower serum creatinine and higher serum calcium levels in the high-dose group.

In females, glucose levels were statistically significantly increased in all dose groups. In mid-dose females, the alkaline phosphatase activity was significantly higher and the cholesterol level lower than in the control groups. In the high-dose females, chloride levels were statistically significantly increased.

In mid-dose males, statistically significantly higher absolute and relative prostate weight, and lower relative brain weights were observed compared to the controls. In the mid-dose female group, statistically significantly higher absolute, but not relative thymus weights were observed.

[16] Annex 7.01.
[17] Annex 7.02.
[18] Annex 7.03.

Overall, the Panel noted that the differences to controls in haematological and clinical chemistry parameters or in organ weights were without apparent dose–response relationship and the values were within historical control ranges from the laboratory. Additionally, no treatment-related microscopical changes were seen in organs for which the differences in absolute or relative weights were reported. Accordingly, the Panel concluded that the no observed adverse effect level (NOAEL) in this study was 1,400 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 the α-amylase produced with the genetically modified *A. niger* strain NZYM-MC was assessed by comparing its amino acid sequence with those of known allergens according to the scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms (EFSA GMO Panel, 2010). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, two matches were found. The matching allergens are Asp o 21, an α-amylase produced by *Aspergillus oryzae* and Sch c 1, a glucoamylase produced by *Schizophyllum commune*.

Both glucoamylase from *S. commune* (Toyotome et al., 2014) and α-amylase from *A. oryzae* (Brisman and Belin, 1991; Sander et al., 1998; Brisman, 2002; Quirce et al., 2002) are known as occupational respiratory allergens associated with baker's asthma. However, several studies have shown that adults with occupational asthma to a food enzyme (as described for α-amylase from *A. oryzae*) can ingest respiratory allergens without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Poulsen, 2004; Armentia et al., 2009). Considering the wide use of α-amylase as a food enzyme, only a low number of case reports has been described in the literature focused on allergic reactions upon oral exposure to α-amylase in individuals respiratory sensitised to α-amylase (Losada et al., 1992; Quirce et al., 1992; Baur and Czuppon, 1995; Kanny and Moneret-Vautrin, 1995; Moreno-Ancillo et al., 2004). Such information has not been reported for glucoamylase.

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

The Panel noted that an allergic reaction upon oral ingestion of this α-amylase produced with the genetically modified *A. niger* strain NZYM-MC, in individuals respiratory sensitised to α-amylase or to glucoamylase cannot be ruled out, 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 No 1169/2011)[19] 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.

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, distilled alcohol production and baking processes at the recommended use levels summarised in Table 2.

[19] 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.

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	407 mg TOS/kg starch (up to 200 FAU(F)/kg of starch dry matter)
Distilled alcohol production	Starch dry matter	407 mg TOS/kg starch (up to 200 FAU(F)/kg of starch dry matter)
Baking processes	Flour	318 mg TOS/kg flour (up to 160 FAU(F)/kg of flour)

TOS: total organic solids; FAU(F): Fungal α-Amylase 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 intended use in 'Cereal-based processes.' In addition, the applicant changed the use level of the food enzyme in 'Baking process' from up to 200 FAU(F)/kg starch dry matter to up to 160 FAU(F)/kg flour.
(c): Technical dossier: p. 59. and Additional information received in October 2017.

In starch processing, the food enzyme is typically added during the saccharification step where it degrades gelatinised starch into dextrins. The α-amylase can also be used for raw starch hydrolysis where the starch is not completely gelatinised.

In distilled alcohol production, the food enzyme is typically applied during the pre-saccharification together with other saccharification enzymes (e.g. glucoamylase) to degrade the dextrins to fermentable sugars. In plants using the simultaneous saccharification and fermentation (SSF) process, liquefied mash is pumped into the fermenter, where the α-amylase and other saccharification enzymes are added together with yeast at the beginning of fermenter fill.

Experimental data have been provided on the removal (> 99%) of protein in the course of distilled alcohol production and 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 distillation and the purification steps applied during the production of glucose syrups, i.e. filtration, ion exchange chromatography, carbon treatment.

In baking processes, the food enzyme is added to flour during the preparation of dough. The α-amylase hydrolyses starch from granules that have been damaged during milling and release fermentable sugars and dextrins. This reaction shortens the processing time and decreases dough viscosity. The latter facilitates the handling of the dough, resulting in more uniform products with better properties (increased firmness, reduced oil absorption and less stockiness).

The food enzyme remains in the dough. Based on data provided on thermostability (see Section 3.3.1), it is anticipated that the α-amylase is inactivated during baking processes.

3.5.2. Dietary exposure estimation

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

For the baking 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[20] 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

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

population and per age class. Surveys with only one day per subject were excluded and high-level exposure/intake was calculated for only those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011).

Table 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)	0.059–0.884 (10)	0.671–1.903 (14)	0.763–1.838 (19)	0.417–1.171 (18)	0.313–0.730 (19)	0.310–0.647 (18)
Min–max 95th percentile (number of surveys)	0.347–3.784 (8)	1.674–3.238 (12)	1.497–3.451 (19)	0.932–2.385 (17)	0.687–1.429 (19)	0.620–1.131 (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 (1,400 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates of 0.059–1.903 mg/kg bw per day at the mean and from 0.347 to 3.784 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposures (MOEs) above 370.

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 baking processes, the Panel concludes that the food enzyme α-amylase produced with the genetically modified *A. niger* strain NZYM-MC 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 'Alpha-amylase produced by a genetically modified strain of Aspergillus niger (strain NZYM-MC).' August 2013. Submitted by Novozymes A/S.
2) Additional information submitted in March 2015 by the applicant.
3) Additional information submitted in October 2017 by the applicant.
4) Additional information on 'Food enzyme removal during the production of cereal-based distilled alcoholic beverages' and '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 February 2018 by the applicant.
6) Summary report on genotoxicity, subchronic toxicity study and allergenicity for α-amylase produced with a genetically modified strain of *Aspergillus niger* (strain NZYM-MC) by Novozymes A/S. Delivered by FoBiG GmbH (Freiburg, Germany) on 6 August 2014.

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Abbreviations

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
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen GMbH
EC	Enzyme Commission
EINECS	European Inventory of Existing Commercial Chemical Substances

FAO	Food and Agricultural Organization
FAU(F)	Fungal α-Amylase Units (F standard)
[redacted]	[redacted]
FOA	5-fluoro-orotic acid
[redacted]	[redacted]
GLP	Good Laboratory Practice
GM	genetically modified
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
LOD	limit of detection
MOE	margin of Exposure
NOAEL	no observed adverse effect level
ORI	origin of replication initiation
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
SSF	simultaneous saccharification and fermentation
SDS–PAGE	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	total organic solids
UTR	untranslated region
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).