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Safety evaluation of the food enzyme α -amylase from *Aspergillus oryzae* (strain DP-Bzb41)

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Abstract

The food enzyme α -amylase (4- α -D-glucan glucanohydrolase, EC 3.2.1.1) is produced with a non-genetically modified *Aspergillus oryzae* (strain DP-Bzb41) by Danisco US Inc. (USA). The α -amylase food enzyme is intended to be used in baking, brewing, distilled alcohol production and starch processing for the glucose syrup production. Based on the maximum use levels for baking and brewing processes and individual data from the EFSA Comprehensive European Food Database, dietary exposure to the food enzyme–Total Organic Solids (TOS) was estimated to be up to 2.59 mg TOS/kg body weight (bw) per day. Since residual amounts of TOS are removed during distilled alcohol production and by the purification steps applied during starch processing, dietary exposure for these processes was not calculated. 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 rats. The Panel identified a no observed adverse effect level (NOAEL) of 1,000 mg TOS/kg bw per day, the highest dose tested. Comparison with the estimated dietary exposure, results in a margin of exposure of at least 386. Similarity of the amino acid sequence to those of known allergens was searched and one match to respiratory allergen was found (an amylase from another strain of *A. oryzae*). The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but the likelihood is considered to be low. Based on the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

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1. Introduction

Article 3 of the Regulation (EC) No 1332/2008¹ provides definition 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 1332/2008¹ 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 1331/2008² established the European Union (EU) procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

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

The 'Guidance on submission of a dossier on food enzymes for safety evaluation' (EFSA 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 European Union (EU) Community list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7(2) of Regulation (EC) No 1332/2008¹ on food enzymes.

Five applications have been introduced by the company 'Danisco US Inc.' for the authorisation of the food enzymes glucan 1,4- α -glucosidase from a genetically modified strain of *Trichoderma reesei* (DP-Nzh63), subtilisin from a genetically modified strain of *Bacillus subtilis* (DP-Ezx62), subtilisin from a genetically modified strain of *Bacillus subtilis* (DP-Ezx42), α -amylase from *Aspergillus oryzae* (DP-Bzb41), and glucan 1,4- α -glucosidase from a genetically modified strain of *Trichoderma reesei* (DP-Nzh38).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011³ implementing Regulation (EC) No 1331/2008,² the Commission has verified that the five applications fall within the scope of the food enzyme Regulation and contain all the elements required under Chapter II of that Regulation.

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

² Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, pp. 1–6.

³ Commission Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 64, 11.3.2011, p. 15–24.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes glucan 1,4- α -glucosidase from a genetically modified strain of *Trichoderma reesei* (strain DP-Nzh63), subtilisin from a genetically modified strain of *Bacillus subtilis* (strain DP-Ezx62), subtilisin from a genetically modified strain of *Bacillus subtilis* (strain DP-Ezx42), α -amylase from *Aspergillus oryzae* (strain DP-Bzb41), and glucan 1,4- α -glucosidase from a genetically modified strain of *Trichoderma reesei* (strain DP-Nzh38) in accordance with Article 17.3 of Regulation (EC) No 1332/2008¹ on food enzymes.

1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of food enzyme α -amylase from a non-genetically modified microorganism *Aspergillus oryzae* (strain DP-Bzb41).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme α -amylase from a non-genetically modified microorganism *A. oryzae* (strain DP-Bzb41).

Additional information was requested from the applicant during the assessment process on 23 May 2019 and was consequently provided (see 'Documentation provided to EFSA').

2.2. Methodologies

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

The current 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA CEF Panel, 2009) has been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance to the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016).

3. Assessment⁴

IUBMB nomenclature: alpha-Amylase

Systematic name: 4- α -D-glucan glucanohydrolase

Synonyms: Amylase; Glycogenase; Endo-amylase; Taka-amylase A; 1,4-alpha-D-glucan glucanohydrolase

IUBMB No.: 3.2.1.1

CAS No.: 9000-90-2

EINECS No.: 232-565-6

The α -amylase catalyses the hydrolysis of (1 \rightarrow 4)- α -D-glucosidic linkages in polysaccharides (amylose and amylopectin), resulting in the generation of oligosaccharides. It is intended to be used in baking, brewing, distilled alcohol production, and starch processing for the glucose syrup production.

3.1. Source of the food enzyme⁵

The food enzyme α -amylase is produced with a non-genetically modified filamentous fungus *A. oryzae* (strain DP-Bzb41). It is deposited in the collection of [REDACTED] with deposition number [REDACTED].⁶

The production strain has an extended genealogy, arising from an initial isolate made in Japan in the 1900s. This initial isolate has, in subsequent years, been subjected to [REDACTED] intended to improve its fermentation characteristics and enzyme yield.⁷

⁴ Technical dossier/2nd submission/p. 36.

⁵ Technical dossier/2nd submission/p. 43–48.

⁶ Technical dossier/Additional data, 23 August 2019/Annex U.

⁷ Technical dossier/Additional data, 23 August 2019/Annex Y.

Evidence of the identity of the fungal strain used in the production of the food enzyme based on a phylogenomic approach was provided.⁸ A maximum likelihood phylogenetic tree based on the [REDACTED] showed that the production strain is firmly located within the *A. flavus* and *A. oryzae* clade.

3.2. Production of the food enzyme⁹

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004,¹⁰ with food safety procedures based on Hazard Analysis and Critical Control Points (HACCP¹¹), 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, batch or 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 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 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 food enzyme α -amylase consists of a single polypeptide chain of [REDACTED] amino acids.¹² The molecular mass of the food enzyme, calculated from its amino acid sequence is [REDACTED] kDa.¹³ Analysis by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis¹⁴ for the three batches for commercialisation and the batch used for toxicological testing showed a single major protein band corresponding to an apparent molecular mass of about [REDACTED] kDa protein. No other enzymatic side activities were reported.¹⁵

The in-house determination of α -amylase activity is based on the hydrolysis of a non-reducing-end blocked *p*-nitrophenyl maltoheptaoside (BPNPG7) substrate in the presence of excess levels of α -glucosidase and glucoamylase and is expressed in Sandstedt Kneen Blish Units (SKBU)/g. One SKBU is defined as the amount of enzyme required to release 1 μ mol of *p*-nitrophenol per minute from BPNPG7 under the conditions described for the assay (pH 5.6, 25°C, reaction time 5 min).¹⁶

The food enzyme has a temperature maximum around 50°C (pH 5.6)¹⁷ and a pH maximum around pH 5 (40°C).¹⁸ Thermostability was tested by the pre-incubation of the food enzyme at 55°C and at a pH of 4.6 for periods up to 140 min. Enzyme activity was lost after incubation for 20 min under these conditions.¹⁷

3.3.2. Chemical parameters¹⁹

Data on the chemical parameters of the food enzyme were provided for four food enzyme batches, three batches used for commercialisation and one batch produced for the toxicological tests (Table 1). The average Total Organic Solids (TOS) of the three food enzyme batches for commercialisation was 29.73%. The average enzyme activity/mg TOS ratio of the three food enzyme batches for commercialisation is 304 SKBU/mg TOS.

⁸ Technical dossier/Additional data, 23 August 2019/Annex X.

⁹ Technical dossier/2nd submission/p. 49–57; Technical dossier/1st submission/Annex K; Technical dossier/Additional data, 23 August 2019/Annex T.

¹⁰ Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of food additives. OJ L 226, 25.6.2004, pp. 3–21.

¹¹ Technical dossier/1st submission/Annex J.

¹² Technical dossier/1st submission/Annex H.

¹³ Technical dossier/Additional data, 23 August 2019/Annex 1/p. 1.

¹⁴ Technical dossier/2nd submission/p. 37; Technical dossier/1st submission/Annex E; Technical dossier/Additional data, 23 August 2019/Annex W.

¹⁵ Technical dossier/2nd submission/p. 40–41.

¹⁶ Technical dossier/1st submission/Annex D.

¹⁷ Technical dossier/2nd submission/p. 42.

¹⁸ Technical dossier/2nd submission/p. 41.

¹⁹ Technical dossier/2nd submission/p. 37; Technical dossier/2nd submission/Annex G; Technical dossier/1st submission/Annex F; Annex O; Annex C.

Table 1: Compositional data of the food enzyme

Parameter	Unit	Batches			
		1	2	3	4 ^{(a),(d)}
α -Amylase activity	SKBU/g batch ^(b)	74,416	105,318	92,634	111,711
Protein	%	23.16	29.47	26.73	35.76
Ash	%	0.36	0.37	0.75	0.68
Water	%	73.63	66.81	68.88	63.40
Total Organic Solids (TOS) ^(c)	%	26.01	32.82	30.37	35.92
α -Amylase activity/mg TOS	SKBU/mg TOS	286	321	305	311

(a): Batch used for the toxicological studies.

(b): SKBU/g: Sandstedt Kneen Blish Unit/g (see Section 3.3.1).

(c): TOS calculated as 100% - % water - % ash.

(d): Technical dossier/2nd submission/p. 73; Technical dossier/1st submission/Annex O.

3.3.3. Purity

The lead content²⁰ in the three commercial batches and in the batch 4 used for toxicological studies complies with the specification for lead (≤ 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, mercury and cadmium in the batch used for toxicological testing were each below the limits of detection (LODs) of the employed methodologies.²¹

The food enzyme α -amylase 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* species²² are absent in 25 g of sample and total coliforms should not exceed 30 colony forming units (CFU) per gram. No antimicrobial activity was detected in any of these batches²³ (FAO/WHO, 2006).

The presence of a number of mycotoxins (total aflatoxin, aflatoxin B1, zearalenone, ochratoxin A, sterigmatocystin and fumonisins B1 + B2) was examined in the commercial batches²³ and batch used for toxicological testing.²⁴ All were found to be below the limits of detection²⁵ of the applied analytical methods.

Strains of *Aspergillus*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Blumenthal, 2004; Frisvad et al., 2018). The applicant did not provide information on other secondary metabolites which maybe produced under the conditions of fermentation which might contribute to the food enzyme TOS. This issue is addressed by the toxicological examination of the food enzyme TOS.

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

3.3.4. Absence of the production strain²⁶

The absence of the production strain in the food enzyme was demonstrated in three independent batches of the food enzyme.

No colonies were produced.

3.4. Toxicological data

A battery of toxicological tests including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian chromosomal aberration test, and a repeated dose 90-day oral toxicity study in rats has been provided. The batch 4 used in these studies is considered representative of the batches intended for commercialisation and thus suitable as a test item.

²⁰ Technical dossier/2nd submission/p. 39; Technical dossier/1st submission/Annex F and G.

²¹ Technical dossier/1st submission/Annex O; LOD: As = 3 mg/kg, Hg = 0.5 mg/kg, Cd = 0.5 mg/kg.

²² Technical dossier/2nd submission/p. 39; Technical dossier/1st submission/Annex O.

²³ Technical dossier/2nd submission/Annex G_updated.

²⁴ Technical dossier/1st submission/Annex O.

²⁵ Technical dossier/1st submission/Annex G_updated; LOD for total aflatoxins and aflatoxin B1 < 5 μ g/kg; LOD for zearalenone < 250 μ g/kg; LOD for ochratoxin A < 10 μ g/kg; LOD for sterigmatocystin < 100 μ g/kg; LOD for fumonisin B1 + B2 < 2,000 μ g/kg.

²⁶ Technical dossier/1st submission/Annex F and Annex G.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was performed according to OECD Test Guideline 471 (OECD, 1997a), and following Good Laboratory Practice (GLP) in four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2uvrA, in the presence or absence of metabolic activation (S9).²⁷ The treat and plate method was applied. Two experiments were carried out in triplicate using five different concentrations of the food enzyme (50, 150, 500, 1,500 and 5,000 μ g total protein per plate, corresponding to 50, 151, 502, 1,507, 5,022 μ g TOS/plate). No evidence of toxicity was observed under any of the conditions tested. Upon treatment with the food enzyme, there was no increase in revertant colony numbers. Therefore, the Panel concluded that the food enzyme α -amylase 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 chromosomal aberration test

The *in vitro* mammalian chromosome aberration test was carried out according to the OECD Test Guideline 473 (OECD, 1997b) and following GLP in cultured human peripheral blood lymphocytes.²⁸ Two experiments were performed. In the first experiment, applying 4 h treatment + 16 h recovery, the cultures were exposed to concentrations of 1,000, 2,500 and 5,000 μ g total protein/mL (corresponding to 1,004, 2,511 and 5,022 μ g TOS/mL), either in the presence or the absence of the S9. In the second experiment, applying continuous 20 h treatment without metabolic activation, the concentrations tested were 1,000, 2,500 and 5,000 μ g total protein/mL (corresponding to 1,004, 2,511 and 5,022 μ g TOS/mL). For all food enzyme concentrations used, the frequency of cells with chromosomal aberrations was similar to that of negative controls. No significant increase in polyploid or endoreplicated cells was observed. The Panel concluded that the food enzyme α -amylase did not induce chromosomal aberrations in cultured human peripheral blood lymphocytes when tested up to 5,000 μ g total protein/mL (corresponding to 5,022 μ g TOS/mL) under the experimental conditions employed for this study.

Therefore, the Panel concluded that on the basis of the *in vitro* studies there is no concern for genotoxicity for the α -amylase tested.

3.4.2. 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.²⁹ Groups of 10 male and 10 female CrI:CD(SD) rats received by gavage the food enzyme in doses of 250, 500 and 1,000 mg TOS/kg body weight (bw) per day. Controls received the vehicle (deionised water).

Two deaths were recorded; one low-dose male (day 57, gross and microscopic findings revealed severe lung and thymus haemorrhage) and one high-dose female (day 87, gross and microscopic findings demonstrated severe lung haemorrhage and pleural haemorrhage) died due to misdosing.

Statistically significant decrease in body weight gain in high-dose males (days 29–36) as compared to controls was recorded. As no other statistically significant differences in body weight or body weight gain in either sex at any interval or time point were observed, the Panel considered this body weight gain change to reflect normal biological variation.

Statistically significant differences in food intake were recorded on several occasions in all treated groups as compared to controls. These differences occurred in opposite directions in males and females, were transient, not reflected in overall food intake (days 1–90) and food efficiency, and did not affect statistically significantly body weight gains. Therefore, these findings were considered by the Panel of not toxicological significance.

Among haematology parameters, a statistically significant decrease in absolute reticulocyte count in high-dose males (14.3%) and in mean corpuscular volume in low-dose females (3.6%) were recorded. These findings were not consistent among genders and considered by the Panel as not treatment-related because of lack of an apparent dose dependency.

There was a statistically significant increase in the prostate weight relative to body weight (29.5%) and relative to brain weight (29.1%) in low-dose males and in relative to body weight (28%) in

²⁷ Technical dossier/1st submission/Annex P; Technical dossier/Additional data, 23 August 2019/Annex V.

²⁸ Technical dossier/1st submission/Annex Q.

²⁹ Technical dossier/1st submission/Annex R.

high-dose males. The Panel considered these findings as incidental in the light of lack of the apparent dose response and microscopic changes in the prostate in these groups.

No other statistically significant differences to controls were observed.

Overall, the Panel identified a no observed adverse effect level (NOAEL) of 1,000 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity³⁰

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 food enzyme α -amylase produced with non-genetically modified microorganism *A. oryzae* strain DP-Bzb41 was assessed by comparing its amino acid sequence¹² with those of known allergens according to the scientific opinion on the assessment of allergenicity of genetically modified plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms (EFSA GMO Panel, 2017). Using higher than 35% identity in a sliding window of 80 amino acids as criterion, one match was found. The matching allergen was TAKA-amylase-A, an α -amylase from *A. oryzae*, which is also referred to as Asp o 21. The sequence identity to this respiratory allergen was 100%.³¹

α -Amylase from *A. oryzae* is not identified as a food allergen by both the AllergenOnline³² and the WHO/IUIS allergen nomenclature sub-committee database.³³ α -Amylase from *A. oryzae* (Brisman and Belin, 1991; Brisman, 2002) is described as an occupational respiratory allergen associated with baker's asthma. However, several studies have shown that adults with occupational asthma to a food enzyme, as described for 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). Considering the wide use of α -amylase, only a low number of case reports have been described focussed 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). The Panel noted that an allergic reaction upon oral ingestion of this α -amylase, produced with non-genetically modified *A. oryzae* (strain DP-Bzb41), in individuals respiratory sensitised to α -amylase 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 or intolerances (Regulation EU No 1169/2011)³⁴ are used as raw materials (██████████)³⁵ 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.

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 four food processes. Intended uses and the recommended use levels are summarised in Table 2.

³⁰ Technical dossier/2nd submission/p. 74–75; Technical dossier/1st submission/Annex H and Annex N; Technical dossier/Additional data, 23 August 2019/Annex T.

³¹ Technical dossier/1st submission/Annex N.

³² <http://www.allergenonline.org/>

³³ <http://allergen.org/>

³⁴ 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.

³⁵ Technical dossier/Additional data, 23 August 2019/Annex T.

³⁶ Technical dossier/2nd submission/p. 58–64.

Table 2: Intended uses and recommended use levels of the food enzyme as provided by the applicant³⁶

Food manufacturing process ^(a)	Raw material	Recommended dosage of the food enzyme
Baking process	Flour	1–10 mg TOS/kg flour
Brewing process	Cereals	55.8–558 mg TOS/kg cereal
Distilled alcohol production	Cereals	23.2–232 mg TOS/kg cereal
Starch processing for the production of glucose syrup	Starch	55.8–558 mg TOS/kg starch

TOS: total organic solids.

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 releases 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).

In brewing processes, the food enzyme is added during the mashing and cereal cooking steps. The α -amylase is used to convert liquefied starch into a maltose-rich solution, improving the amounts of fermentable sugars and thus increasing brewing yield.

In distilled alcohol production, the food enzyme is added during the slurry mixing step, in the liquefaction step and if needed in the pre-saccharification step. α -Amylase is intended to be used to convert liquefied starch into a maltose-rich solution, to increase the amounts of fermentable sugars which results in higher alcohol yields.

In starch processing for the glucose syrup production, the food enzyme is typically added after the liquefaction step where it degrades gelatinised starch into dextrins.

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. 3). The Panel considered the evidence as sufficient to conclude that residual amounts of TOS (including substances other than proteins) are removed by distillation. In addition, taking into account the purification steps applied to the production of glucose syrups, i.e. filtration, ion exchange chromatography, treatment with active carbon, the Panel also considers that the amount of TOS in the final glucose syrup will be removed to a similar degree.

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 and 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 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 Section 3.5.1/Table 2) with the relevant FoodEx categories (Annex B in EFSA CEF Panel, 2016), based on individual consumption data. Exposure from individual 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 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 TOS/kg body weight per day)					
	Infants	Toddlers	Children	Adolescents	Adults	The elderly
Age range	3–11 Months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0.002–0.028 (10)	0.022–0.060 (14)	0.027–0.058 (19)	0.016–0.127 (18)	0.055–0.591 (19)	0.028–0.297 (18)
Min–max 95th percentile (number of surveys)	0.011–0.119 (8)	0.053–0.102 (12)	0.047–0.109 (19)	0.029–0.697 (17)	0.324–2.585 (19)	0.085–1.184 (18)

TOS: total organic solids.

3.5.3. Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the following sources of uncertainties have been considered and are summarised in Table 4.

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

Sources of uncertainties	Direction of impact
Model input data	
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/-
Use of data from food consumption surveys of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Possible national differences in categorisation and classification of food	+/-
Model assumptions and factors	
FoodEx categories included in the exposure assessment were assumed to always contain the food enzyme-TOS	+
Exposure to food enzyme-TOS was always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
Use of recipe fractions in disaggregation FoodEx categories	+/-
Use of technical factors in the exposure model	+/-

FoodEx: a standardised food classification and description system; TOS: total organic solids.

+: 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 (1,000 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates of up to 0.59 mg TOS/kg bw per day at the mean and 2.59 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposure (MOE) of at least 386.

4. Conclusions

Based on the data provided, the removal of TOS during distilled alcohol production and starch processing for the glucose syrup production and the derived margin of exposure for baking and brewing processes, the Panel concluded that the food enzyme α -amylase produced with a non-genetically modified *A. oryzae* (strain DP-Bzb41) does not give rise to safety concerns under the intended conditions of use.

Documentation provided to EFSA

- 1) Technical dossier 'Application for authorisation of Alpha-amylase from *Aspergillus oryzae* (DP-Bzb41) in accordance with Regulation (EC) No 1331/2008' (the first and second submission). 10 March 2015. Submitted by Danisco US Inc. (USA).
- 2) Additional information. 22 August 2019. Submitted by Danisco US Inc. (USA).
- 3) 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 (AMFEP). Unpublished document.

References

- Armentia A, Dias-Perales A, Castrodeza J, Duenas-Laita A, Palacin A and Fernández S, 2009. Why can patients with baker's asthma tolerate wheat flour ingestion? Is wheat pollen allergy relevant? *Allergologia et Immunopathologia*, 37, 203–204.
- Baur X and Czuppon AB, 1995. Allergic reaction after eating α -amylase (Asp o 2)-containing bread. *Allergy*, 50, 85–87.
- Blumenthal CZ, 2004. Production of toxic metabolites in *Aspergillus niger*, *Aspergillus oryzae*, and *Trichoderma reesei*: justification of mycotoxin testing in food grade enzyme preparations derived from the three fungi. *Regulatory Toxicology and Pharmacology*, 39, 214–228. <https://doi.org/10.1016/j.yrtph.2003.09.002>
- Brisman J, 2002. Baker's asthma. *Occupational and Environmental Medicine*, 59, 498–502; quiz 502, 426.
- Brisman J and Belin L, 1991. Clinical and immunological responses to occupational exposure to alpha-amylase in the baking industry. *British Journal of Industrial Medicine*, 48, 604–608.
- Cullinan P, Cook A, Jones M, Cannon J, Fitzgerald B and Taylor AJ, 1997. Clinical responses to ingested fungal alpha-amylase and hemicellulase in persons sensitized to *Aspergillus fumigatus*? *Allergy*, 52, 346–349.
- EFSA (European Food Safety Authority), 2006. Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment. *EFSA Journal* 2006;5(1):438, 54 pp. <https://doi.org/10.2903/j.efsa.2007.438>
- EFSA (European Food Safety Authority), 2009. Guidance of EFSA prepared by the Scientific Committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: General principles. *EFSA Journal* 2009;7(5):1051, 22 pp. <https://doi.org/10.2093/j.efsa.2009.1051>
- EFSA (European Food Safety Authority), 2011. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. *EFSA Journal* 2011;9(3):2097, 34 pp. <https://doi.org/10.2903/j.efsa.2011.2097>
- EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2009. Guidance of the Scientific Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) on the Submission of a Dossier on Food Enzymes for Safety Evaluation by the Scientific Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids. *EFSA Journal* 2009;7(8):1305, 26 pp. <https://doi.org/10.2903/j.efsa.2009.1305>. Updated on May 2013.
- EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), Silano V, Bolognesi C, Castle L, Cravedi J-P, Fowler P, Franz R, Grob K, Gurtler R, Husøy T, Karenlampi S, Mennes W, Milana MR, Penninks A, Smith A, Tavares Pocas MF, Tlustos C, Wolffe D, Zorn H, Zugravu C-A, Arcella D, Liu Y and Engel K-H, 2016. Panel statement on the exposure assessment of food enzymes. *EFSA Journal* 2016;14(11):4581, 9 pp. <https://doi.org/10.2903/j.efsa.2016.4581>
- EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Birch AN, Casacuberta J, De Schrijver A, Gralak MA, Guerche P, Jones H, Manachini B, Messean A, Nielsen EE, Nogue F, Robaglia C, Rostoks N, Sweet J, Tebbe C, Visioli F, Wal J-M, Eigenmann P, Epstein M, Hoffmann-Sommergruber K, Koning F, Lovik M, Mills C, Moreno FJ, van Loveren H, Selb R and Fernandez Dumont A, 2017. Guidance on allergenicity assessment of genetically modified plants. *EFSA Journal* 2017;15(5):4862, 49 pp. <https://doi.org/10.2903/j.efsa.2017.4862>
- FAO/WHO (Food and Agriculture Organization of the United States/World Health Organization), 2006. General specifications and considerations for enzyme preparations used in food processing in Compendium of food additive specifications. 67th meeting. FAO JECFA Monographs 3, 63–67. Available online: <ftp://ftp.fao.org/docrep/fao/009/a0675e/a0675e00.pdf>
- Frisvad JC, Møller LLH, Larsen TO, Kumar R and Arnau J, 2018. Safety of the fungal workhorses of industrial biotechnology: update on the mycotoxin and secondary metabolite potential of *Aspergillus niger*, *Aspergillus oryzae*, and *Trichoderma reesei*. *Applied Microbiology and Biotechnology*, 102, 9481–9515. <https://doi.org/10.1007/s00253-018-9354-1>
- Kanny G and Moneret-Vautrin DA, 1995. Alpha-amylase contained in bread can induce food allergy. *Journal of Allergy and Clinical Immunology*, 95(1 Pt 1), 132–133.
- Losada E, Hinojosa M, Quirce S, Sanchez-Cano M and Moneo I, 1992. Occupational asthma caused by alpha-amylase inhalation: clinical and immunologic findings and bronchial response patterns. *Journal of Allergy and Clinical Immunology*, 89(1 Pt 1), 118–125.

- Moreno-Ancillo A, Dominguez-Noche C, Gil-Adrados AC and Cosmes PM, 2004. Bread eating induced oral angioedema due to alpha-amylase allergy. *Journal of Investigational Allergology and Clinical Immunology*, 14, 346–347.
- OECD (Organisation for Economic Co-operation and Development), 1997a. Bacterial Reverse Mutation Test. Guideline 471, adopted 21.07.1997. Available online: http://www.oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en;jsessionid=9zfgzu35paaq.x-oecd-live-01
- OECD (Organisation for Economic Co-operation and Development), 1997b. Test No. 473: *In Vitro* Mammalian Chromosome Aberration Test), OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing. 21 July 1997. Available online: <https://doi.org/10.1787/9789264071261-en>
- OECD (Organisation for Economic Co-operation and Development), 1998. Repeated Dose 90-day Oral Toxicity Study in Rodents. Guideline 408, adopted 21.09.1998. Available online: http://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en
- Poulsen LK, 2004. Allergy assessment of foods or ingredients derived from biotechnology, gene-modified organisms, or novel foods. *Molecular Nutrition and Food Research*, 48, 413–423. <https://doi.org/10.1002/mnfr.200400029>
- Quirce S, Cuevas M, Díez-Gómez M, Fernández-Rivas M, Hinojosa M, González R and Losada E, 1992. Respiratory allergy to Aspergillus-derived enzymes in baker's asthma. *Journal of Allergy and Clinical Immunology*, 90(6 Pt 1), 970–978.

Abbreviations

AMFEP	Association of Manufacturers and Formulators of Enzyme Products
BNPNG7	<i>p</i> -nitrophenyl maltoheptaoside
bw	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
CFU	colony forming units
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
FoodEx	The food classification and description system
HACCP	Hazard Analysis and Critical Control Points
GLP	Good Laboratory Practice
GM	genetically modified
GMO	genetically modified organism
GMP	Good Manufacturing Practice
IUBMB	International Union of Biochemistry and Molecular Biology
IUIS	International Union of Immunological Societies
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
MOE	margin of exposure
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
■	■
SDS-PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
S9	metabolic activation
SKBU	Sandstedt Kneen Blish Unit
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 <https://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2019.5899/supinfo>).

The file contains two sheets, corresponding to two tables.

Table 1: Average and 95th percentile exposure to the food enzyme–TOS per age class, country and survey.

Table 2: Contribution of food categories to the dietary exposure to the food enzyme–TOS per age class, country and survey.

Appendix B – Population groups considered for the exposure assessment

Population	Age range	Countries with food consumption surveys covering more than 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).