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Safety evaluation of the food enzyme α-amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb25)

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Abstract

The food enzyme α-amylase (4-α-D-glucan glucanhydrolase; EC 3.2.1.1) is produced with the genetically modified strain *Bacillus licheniformis* DP-Dzb25 by Danisco US Inc. It is intended to be used in distilled alcohol production, starch processing for the production of glucose syrups, and in brewing processes. Since residual amounts of the food enzyme are removed by distillation and during starch processing, no dietary exposure was calculated for these food processes. Based on the maximum use levels recommended for 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 0.138 mg TOS/kg body weight (bw) per day. The production strain of the food enzyme contains multiple copies of a known antimicrobial resistance gene and consequently, it does not fulfil the requirements for the Qualified Presumption of Safety (QPS) approach to safety assessment. However, considering the absence of viable cells and DNA from the production organism in the food enzyme, this is not considered to be a risk. As no other concerns arising from the microbial source and its subsequent genetic modification or from the manufacturing process have been identified, the Panel considers that toxicological tests are not needed for the assessment of this food enzyme. Similarity of the amino acid sequence to those of known allergens was searched for and no match was found. The Panel notes that the food enzyme may contain a known allergen. Therefore, allergenicity cannot be excluded for uses other than distilled alcohol production. Apart from potential allergenicity, the Panel concluded that the food enzyme 4-α-D-glucan glucanhydrolase produced with the genetically modified *B. licheniformis* strain DP-Dzb25 does not give rise to safety concerns under the intended conditions of use.

Keywords: food enzyme, α-amylase, 1,4-α-D-glucan glucanohydrolase, EC 3.2.1.1, *Bacillus licheniformis*, DP-Dzb25, genetically modified microorganism

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1. Introduction

Article 3 of the Regulation (EC) No 1332/2008[1] 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 1332/2008 on food enzymes came into force. This Regulation applies to enzymes that are added to food to perform a technological function in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food, including enzymes used as processing aids. Regulation (EC) No 1331/2008[2] established 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, 2009a) lays down the administrative, technical and toxicological data required.

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background as provided by the European Commission

Only food enzymes included in the 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 1332/2008 on food enzymes.

Five applications have been introduced by the companies "BASF Enzymes LLC1" for the authorisation of the food enzyme Alpha-amylase from a genetically modified strain of *Pseudomonas fluorescens* (BD15754), "DSM Food Specialties B.V." for the authorisation of the food enzyme Phospholipase C from a genetically modified strain *of Pichia pastoris* (PRF), and "Danisco US Inc." for the authorisation of the food enzymes Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb25), Xylose isomerase from a genetically modified strain of *Streptomyces rubiginosus* (DP-Pzn37), and Alpha-amylase from a genetically modified strain of *Bacillus amyloliquefaciens* (DP-Czb53).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008, 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 Alpha-amylase from a genetically modified strain of *Pseudomonas fluorescens* (strain BD15754), Phospholipase C from a genetically modified strain of *Pichia pastoris* (strain PRF), Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (strain DP-Dzb25), Xylose isomerase from a genetically modified strain of *Streptomyces rubiginosus* (strain DP-Pzn37), and Alpha-amylase from a genetically modified strain of *Bacillus amyloliquefaciens* (strain DP-Czb53) 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 food enzyme α-amylase from a genetically modified *Bacillus licheniformis* (DP-Dzb25).

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 obtained from a genetically modified *B. licheniformis* (strain DP-Dzb25).

Additional information was sought from the applicant during the assessment process in response to requests from EFSA sent on 8 March 2019 and was subsequently provided (see 'Documentation provided to EFSA').

Following the reception of the additional data, EFSA requested a clarification teleconference, which was held on 23 September 2019; after which the applicant provided additional data on 23 September 2019.

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA Guidance on transparency in the scientific aspects of risk assessment (EFSA, 2009b) as well as in the Statement on characterisation of microorganisms used for the production of food enzymes (EFSA CEP Panel, 2019) and following the relevant existing guidance's of EFSA Scientific Committees.

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

3. Assessment

IUBMB nomenclature:	α-amylase
Systematic name:	4-α-D-glucan glucanhydrolase
Synonyms:	Glycogenase, endoamylase, 1,4-α-D-glucan glucanohydrolase, Taka-amylase
IUBMB No:	EC 3.2.1.1
CAS No:	9000-90-2
EINECS No:	232-565-6.

The 4-α-D-glucan glucanhydrolase catalyses the hydrolysis of 1,4-α-glucosidic linkages in starch (amylose and amylopectin), glycogen and related polysaccharides and oligosaccharides, resulting in the generation of soluble dextrins and other malto-oligosaccharides. It is intended to be used in starch processing for the production of glucose syrup, brewing and distilled alcohol production.

3.1. Source of the food enzyme

The 4-α-D-glucan glucanhydrolase is produced with a genetically modified strain of *B. licheniformis* (DP-Dzb25), which is deposited in the Westerdijk Fungal Biodiversity Institute (The Netherlands), with deposition number [redacted][4]

[4] Technical dossier/Additional information August 2019/Annex AB.

The species *B. licheniformis* is included in the list of organisms for which the Qualified Presumption of Safety (QPS) may be applied, provided that the absence of acquired antimicrobial resistance genes and toxigenic activity are verified for the specific strain used (EFSA BIOHAZ Panel, 2017). The parental strain was identified as *B. licheniformis* by ribotyping of rRNA genes and by 16S rRNA gene sequence analysis.[5] The absence of cytotoxicity activity in the production strain was confirmed using CHO_K1 (Chinese hamster ovary cells).[6] It contains a [redacted] resistance gene ([redacted]), [redacted] The strain was not tested for other antimicrobial resistance determinants. Therefore, the production strain cannot be considered to qualify for the QPS status.

3.1.1. Characteristics of the parental and recipient microorganisms

[redacted][7]

3.1.2. Characteristics of the inserted sequences

[redacted][8]

3.1.3. Description of the genetic modification process[7]

[redacted][9][10]

3.1.4. Safety aspects of the genetic modification

[redacted][11]

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[12], with food safety procedures based on Hazard Analysis and Critical Control Points and in accordance with current Good Manufacturing Practice.

[5] Technical dossier/1st submission/Annex S.
[6] Technical dossier/Additional information August 2019/Annex AK.
[7] Technical dossier/2nd submission/Annex U.
[8] Technical dossier/1st submission/Annex I.
[9] Technical dossier/Additional information August 2019/Annex AC.
[10] Technical dossier/Additional information August 2019/Annex AD.
[11] Technical dossier/2nd submission/Annex AA.
[12] 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.

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 ferment is treated with [redacted] and then the solid biomass 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.[10]

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 4-α-D-glucan glucanhydrolase is a single polypeptide chain of [redacted] amino acids.[8] The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be [redacted] kDa.[13] The food enzyme was analysed by denaturing polyacrylamide gel electrophoresis (PAGE) analysis. The gels showed a single major protein band corresponding to an apparent molecular mass of about 52 kDa, consistent with the expected mass of the enzyme. No other enzymatic side activities were reported.

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. The released *p*-nitrophenyl is monitored spectrophotometrically (reaction conditions: 25°C, 5 min). The enzyme activity is quantified relative to an internal enzyme standard and expressed in Liquifon Units/g (LU/g).[14]

The food enzyme has a temperature optimum around 80°C (pH 5.6) and a pH optimum around pH 6 (90°C). Thermostability was tested after a pre-incubation of the food enzyme at 75°C at different times (pH 4.6). Enzyme activity decreased by 90% after 10 min of pre-incubation, with no activity detected after 30 min.

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three food enzyme batches (Table 1).[15] The average total organic solids (TOS) of the three food enzyme batches for commercialisation was 8.08%. The average enzyme activity/TOS ratio of the three food enzyme batches for commercialisation is 401 LU/mg TOS.

Table 1: Compositional data of the food enzyme

Parameter	Unit	Batches		
		1	2	3
4-α-D-glucan glucanhydrolase activity	LU/g batch(a)	31,225	35,063	28,998
Protein	% (w/w)	5.26	7.07	4.52
Ash	% (w/w)	1.47	1.71	1.22
Water	% (w/w)	90.89	88.21	92.27
Total organic solids (TOS)(b)	% (w/w)	7.64	10.08	6.51
4-α-D-glucan glucanhydrolase activity/mg TOS	LU/mg TOS	408.16	348.43	446.4

(a): Units (see Section 3.3.1).
(b): TOS calculated as 100% – % water – % ash.

[13] Technical dossier/Additional information August 2019.
[14] Technical dossier/1st submission/Annex E.
[15] Technical dossier/Additional information August 2019/Annex AI.

3.3.3. Purity

The lead content in three commercial batches was below 5 mg/kg,[15,16] which 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).

The food enzyme complies with the microbiological criteria as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006), which stipulate that *Escherichia coli* and *Salmonella* species are absent in 25 g of sample and total coliforms are not more than 30 colony forming units (CFU) per gram.[15] No antimicrobial activity was detected in any of these batches according to the levels specified in (FAO/WHO, 2006).[15,17]

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 [redacted][18]

The absence of recombinant DNA in the food enzyme was demonstrated [redacted][19]

3.4. Toxicological data

No toxicological tests were provided by the applicant.

Although all other requirements for the QPS have been met, the production strain carries multiple copies of an acquired antimicrobial resistance gene and therefore cannot be considered as suitable for the QPS approach. However, no risk is expected from the presence of this antimicrobial resistance gene in the production strain, as the enzyme has been shown not to contain viable cells and DNA (Section 3.3.4). As no other concerns arising from the microbial source and its subsequent genetic modification or from the manufacturing process have been identified, the Panel considers that toxicological tests are not needed for the assessment of this food enzyme.

3.5. 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 4-α-D-glucan glucanhydrolase produced with the genetically modified *B. licheniformis* strain DP-Dzb25 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 TAKA-amylase-A from *Aspergillus oryzae.*

No information is available on oral and respiratory sensitisation or elicitation reactions of this 4-α-D-glucan glucanhydrolase.

The enzyme α-amylase from *A. oryzae* (Brisman and Belin, 1991; Sander et al., 1998; Brisman, 2002) is known as 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 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 have 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).

[16] LOD: 0.05 mg/kg.
[17] LOD: 1 mL.
[18] Technical dossier/Additional information August 2019/Annex AF.
[19] Technical dossier/Additional information August 2019/Annex AG.

According to the information provided, substances or products that may cause allergies or intolerances (Regulation (EU) No 1169/2011[20]) are used as raw materials ([redacted]) in the media fed to the microorganisms. However, during the fermentation process, these products will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the microbial biomass and fermentation solids are removed. Taking into account 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 also notes that [redacted] a known allergen, is used during the downstream processing of the food enzyme, and is likely to be present in the final product.

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 as in the case of distilled alcohol production. 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 food enzyme remains in the beer.

The Panel considered that, under the intended conditions of use (other than distilled alcohol production), the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme cannot be excluded, in particular due to the potential presence of [redacted]

3.6. Dietary exposure

3.6.1. Intended use of the food enzyme

The food enzyme is intended to be used in three food manufacturing 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

Food manufacturing process[(a)]	Raw material	Recommended dosage of the food enzyme
Brewing processes	Cereals	Up to 30 mg TOS/kg cereals
Distilled alcohol production	Cereals	Up to 30 mg TOS/kg cereals
Starch processing for the production of glucose syrups	Starch	Up to 30 mg TOS/kg starch

TOS: total organic solids.

(a): The description provided by the applicant has been harmonised by EFSA according to the 'EC working document describing the food processes in which food enzymes are intended to be used' – not yet published at the time of adoption of this opinion.

In starch processing, the food enzyme is typically added during the saccharification step where it degrades gelatinised starch into dextrins. The 4-α-D-glucan glucanhydrolase can also be used for raw starch hydrolysis where the starch is not completely gelatinised.

In distilled alcohol production, 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 process, liquefied mash is pumped into the fermenter, where the 4-α-D-glucan glucanhydrolase and other saccharification enzymes are added together with yeast at the beginning of fermenter fill.

Experimental data have been provided showing 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 (including substances other than proteins) are removed by distillation. In addition, taking into account the purification steps applied to the production of glucose syrups, the Panel also considers that the amount of TOS in the final glucose syrup will be removed to a similar degree.

In brewing processes, the food enzyme is added at the beginning of the mashing step with other saccharification enzymes (β-amylase) to degrade starch to fermentable sugars. The food enzyme

[20] 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.

remains in the beer. Based on data provided on thermostability (see Section 3.3.1), it is expected that the 4-α-D-glucan glucanhydrolase may not be fully inactivated during brewing.

3.6.2. 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 two processes, i.e. distilled alcohols and glucose syrups were excluded from the estimation.

For 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[21] 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 individual FoodEx categories was subsequently summed up, averaged over the total survey period and normalised for body weight. 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 (10)	0 (14)	0.000–0.001 (19)	0.000–0.006 (18)	0.002–0.031 (19)	0.001–0.015 (18)
Min–max 95th percentile (number of surveys)	0 (8)	0 (12)	0 (19)	0.000–0.036 (17)	0.017–0.138 (19)	0.004–0.063 (18)

TOS: total organic solids.

Based on the maximum use levels recommended for brewing processes and individual data from the EFSA Comprehensive European Food Database, dietary exposure to the food enzyme-total organic solids was estimated to be up to 0.138 mg TOS/kg bw per day.

3.6.3. 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.

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

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	+
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.

4. Conclusions

The Panel notes that the food enzyme may contain [redacted], a known allergen. Therefore, allergenicity cannot be excluded for uses other than distilled alcohol production.

Based on the data provided, the Panel concluded that the food enzyme 4-α-D-glucan glucanhydrolase produced with the genetically modified *B. licheniformis* strain DP-Dzb25 does not give rise to other safety concerns under the intended conditions of use.

The production strain of the food enzyme contains multiple copies of known antimicrobial resistance gene. However, based on the absence in the food enzyme of viable cells and DNA from the production organism, this is not considered to be a risk.

Documentation provided to EFSA

1) Application for authorisation of α-amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb25) in accordance with Regulation (EC) No 1331/2008. March 2015. Submitted by Danisco US Inc.
2) Additional information. August 2019. Submitted by Danisco US Inc.
3) Summary report on GMM part for α-amylase produced by *Bacillus licheniformis* strain DP-Dzb25, EFSA-Q-2016-00202. 2018. Delivered by the Technical University of Denmark.
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.

References

Armentia A, Días-Perales A, Castrodeza J, Dueñas-Laita A, Palacin A and Fernándes 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. A case report. Allergy, 50, 85–87.

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 α-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), 2009a. Guidance of the Scientific Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) on the Submission of a Dossier on Food Enzymes for Safety Evaluation by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids. EFSA Journal 2009;7(8):1305, 26 pp. https://doi.org/10.2903/j.efsa.2009.1305

EFSA (European Food Safety Authority), 2009b. Guidance of the Scientific Committee on Transparency in the Scientific Aspects of Risk Assessments carried out by EFSA. Part 2: general Principles. EFSA Journal 2009;7 (5):1051, 22 pp. https://doi.org/10.2903/j.efsa.2009.1051

EFSA (European Food Safety Authority), 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 BIOHAZ Panel (EFSA Panel on Biological Hazards), Ricci A, Allende A, Bolton D, Chemaly M, Davies R, Girones R, Herman L, Koutsoumanis K, Lindqvist R, Nørrung B, Robertson L, Ru G, Sanaa M, Simmons M, Skandamis P, Snary E, Speybroeck N, Ter Kuile B, Threlfall J, Wahlström H, Cocconcelli PS, Klein G (deceased), Prieto Maradona M, Querol A, Peixe L, Suarez JE, Sundh I, Vlak JM, Aguilera-Gómez M, Barizzone F, Brozzi R, Correia S, Heng L, Istace F, Lythgo C and Fernández Escámez PS, 2017. Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA. EFSA Journal 2017;15(3):4664, 177 pp. https://doi.org/10.2903/j.efsa.2017.4664

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), Silano V, Bolognesi C, Castle L, Cravedi JP, Fowler P, Franz R, Grob K, Gürtler R, Husøy T, Kärenlampi S, Mennes W, Milana MR, Penninks A, Smith A, Tavares Poças MF, Tlustos C, Wölfle D, Zorn H, Zugravu CA, Arcella D, Liu Y and Engel KH, 2016. Panel statement on the exposure assessment of food enzymes. EFSA Journal 2016;14 (11):4581, 9 pp. https://doi.org/10.2903/j.efsa.2016.4581

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Silano V, Barat Baviera JM, Bolognesi C, Brüschweiler BJ, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mortensen A, Rivière G, Steffensen I-L, Tlustos C, Van Loveren H, Vernis L, Zorn H, Glandorf B, Herman L, Aguilera J and Chesson A, 2019. Statement on the characterisation of microorganisms used for the production of food enzymes. EFSA Journal 2019;17(6):5741, 13 pp. https://doi.org/10.2903/j.efsa.2019.5741

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010. Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal 2010;8(7):1700, 168 pp. https://doi.org/10.2903/j.efsa.2010.1700

FAO/WHO (Food and Agriculture Organization of the United Nations/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

Kanny G and Moneret-Vautrin DA, 1995. α-Amylase contained in bread can induce food allergy. Journal of Allergy and Clinical Immunology, 95, 132–133.

Losada E, Hinojosa M, Quirce S, Sánchez-Cano M and Moneo I, 1992. Occupational asthma caused by alpha-amylase inhalation: clinical and immunologic findings and bronchial response patterns. The Journal of Allergy and Clinical Immunology, 89, 118–125.

Moreno-Ancillo A, Domínguez-Noche C, Gil-Adrados AC and Cosmes PM, 2004. Bread eating induced oral angioedema due to α-amylase allergy. Journal of Investigational Allergology & Clinical Immunology, 14, 346–347.

Poulsen LK, 2004. Allergy assessment of foods or ingredients derived from biotechnology, gene-modified organisms, or novel food. Molecular Nutrition & Food Research, 48, 413–423.

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 bakers' asthma. The Journal of Allergy and Clinical Immunology, 90, 970–978.

Sander I, Raulf-Heimsoth M, Siethoff C, Lohaus C, Meyer HE and Baur X, 1998. Allergy to *Aspergillus*-derived enzymes in the baking industry: identification of beta-xylosidase from *Aspergillus niger* as a new allergen (Asp n 14). The Journal of Allergy and Clinical Immunology, 102, 256–264.

Abbreviations

CAS	Chemical Abstracts Service
CFU	colony forming units
CHO	Chinese hamster ovary
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GMM	genetically modified microorganism

IUBMB	International Union of Biochemistry and Molecular Biology
MIC	minimum inhibitory concentration
OECD	Organisation for Economic Co-operation and Development
PCR	polymerase chain reaction
QPS	Qualified Presumption of Safety
rRNA	ribosomal ribonucleic acid
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	Total Organic Solids
WGS	whole genome sequence
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://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.5900).

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 dietary exposure of the food enzyme-TOS

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).