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## Safety evaluation of the food enzyme $\alpha$ -amylase from *Bacillus licheniformis* (strain DP-Dzb44)

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### Abstract

The food enzyme  $\alpha$ -amylase (1,4- $\alpha$ -D-glucan glucanohydrolase; EC 3.2.1.1) is produced with the genetically modified *Bacillus licheniformis* strain DP-Dzb44 by Danisco US Inc. The production strain of the food enzyme contains multiple copies of a known antimicrobial resistance gene. However, based on the absence of viable cells and DNA from the production organism in the food enzyme, this is not considered to be a risk. The  $\alpha$ -amylase is intended to be used in distilled alcohol production. Since residual amounts of the food enzyme are removed by distillation, toxicological studies were not considered necessary and no dietary exposure was calculated. Similarity of the amino acid sequence to those of known allergens was searched and one match was found. The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions can be excluded. 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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**Keywords:** food enzyme,  $\alpha$ -amylase, 1, 4- $\alpha$ -D-glucan glucanohydrolase, EC 3.2.1.1, glycogenase, *Bacillus licheniformis*, genetically modified microorganism

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## Table of contents

Abstract.....	1
1. Introduction.....	4
1.1. Background and Terms of Reference as provided by the requestor.....	4
1.1.1. Background as provided by the European Commission.....	4
1.1.2. Terms of Reference.....	5
1.2. Interpretation of the Terms of Reference.....	5
2. Data and methodologies.....	5
2.1. Data.....	5
2.2. Methodologies.....	5
3. Assessment.....	5
3.1. Source of the food enzyme.....	5
3.1.1. Characteristics of the parental and recipient microorganisms.....	6
3.1.2. Characteristics of the introduced sequences.....	6
3.1.3. Description of the genetic modification process.....	6
3.1.4. Safety aspects of the genetic modification.....	6
3.2. Production of the food enzyme.....	7
3.3. Characteristics of the food enzyme.....	7
3.3.1. Properties of the food enzyme.....	7
3.3.2. Chemical parameters.....	7
3.3.3. Purity.....	8
3.3.4. Viable cells and DNA of the production strain.....	8
3.4. Toxicological data.....	8
3.4.1. Allergenicity.....	8
3.5. Dietary exposure.....	9
3.5.1. Intended use of the food enzyme.....	9
3.5.2. Dietary exposure estimation.....	9
4. Conclusions.....	9
Documentation provided to EFSA.....	10
References.....	10
Abbreviations.....	11

## 1. Introduction

Article 3 of the Regulation (EC) No 1332/2008<sup>1</sup> 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 microorganisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.

'Food enzyme preparation' means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

Before January 2009, food enzymes other than those used as food additives were not regulated or were regulated as processing aids under the legislation of the Member States. On 20 January 2009, Regulation (EC) No 1332/2008 on food enzymes came into force. This Regulation applies to enzymes that are added to food to perform a technological function in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food, including enzymes used as processing aids. Regulation (EC) No 1331/2008<sup>2</sup> 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 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 an EU Community 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 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 Association of Manufacturers and Formulators of Enzyme Products (AMFEP) for the authorisation of the food enzyme Bacillolysin from *Bacillus amyloliquefaciens*, and the companies "Danisco US Inc." for the authorisation of the food enzymes Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb44), Beta-galactosidase from a genetically modified strain of *Bacillus subtilis* (DP-Ezg29) and Endo-1,4-beta-xylanase from a genetically modified strain of *Bacillus subtilis* (DP-Ezd31), and "Intertek Scientific & Regulatory Consultancy" for the authorisation of the food enzyme Beta-Fructofuranosidase from *Aspergillus fijiensis* (strain ATCC 20611).

Following the requirements of Article 12.1 of Commission Regulation (EC) No 234/2011<sup>3</sup> implementing Regulation (EC) No 1331/2008<sup>2</sup>, 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.

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

<sup>2</sup> 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

<sup>3</sup> 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 assessment on the food enzymes Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb44), Bacillolysin from *Bacillus amyloliquefaciens*, Beta-galactosidase from a genetically modified strain of *Bacillus subtilis* (DP-Ezg29), Endo-1,4-beta-xylanase from a genetically modified strain of *Bacillus subtilis* (DP-Ezd31), and Beta-Fructofuranosidase from *Aspergillus fijiensis* (strain ATCC 20611) in accordance with the article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

## 1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of food enzyme  $\alpha$ -amylase from a genetically modified *B. licheniformis* (strain DP-Dzb44).

## 2. Data and methodologies

### 2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme  $\alpha$ -amylase from a genetically modified *B. licheniformis* strain DP-Dzb44.

Additional information was requested from the applicant during the assessment process on 19 April 2018 and 19 November 2018 and was consequently provided (see 'Documentation provided to EFSA').

Following the request for additional data sent by EFSA on 19 April 2018, the applicant requested clarification teleconferences, which were held on 16 July 2018 and on 1 October 2018.

### 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) as well as in the EFSA 'Scientific Opinion on Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use' (EFSA GMO Panel, 2011) and following the relevant existing guidance 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. Assessment

IUBMB nomenclature:	$\alpha$ -amylase
Systematic name:	1,4- $\alpha$ -D-glucan glucanohydrolase
Synonyms:	glycogenase
IUBMB No:	EC 3.2.1.1
CAS No:	9000-90-2

The  $\alpha$ -amylase catalyses the hydrolysis of 1,4- $\alpha$ -glucosidic linkages in starch (amylose and amylopectin), glycogen and related polysaccharides and oligosaccharides, resulting in the generation of soluble dextrans and other malto-oligosaccharides. It is intended to be used in distilled alcohol production.

### 3.1. Source of the food enzyme

The  $\alpha$ -amylase is produced with a genetically modified *B. licheniformis* strain DP-Dzb44 [REDACTED] which is deposited in the Westerdijk Fungal Biodiversity Institute (CBS) with the deposit number [REDACTED].<sup>4</sup>

<sup>4</sup> Technical dossier/Additional data March 2019/Annex AC\_SI.

### 3.1.1. Characteristics of the parental and recipient microorganisms

The parental microorganism is the bacterium *B. licheniformis* strain [REDACTED] that was taxonomically identified as *B. licheniformis* by [REDACTED].

The recipient strain *B. licheniformis* [REDACTED] was developed from the parental strain [REDACTED].

### 3.1.2. Characteristics of the introduced sequences

[REDACTED]

### 3.1.3. Description of the genetic modification process

[REDACTED]

### 3.1.4. Safety aspects of the genetic modification

The technical dossier contains all necessary information on the recipient microorganism, the donor organism and the genetic modification process.

The production strain *B. licheniformis* DP-Dzb44 [REDACTED]

[REDACTED]. Its genotypic stability was demonstrated [REDACTED].<sup>9</sup>

<sup>5</sup> Technical dossier/1st submission/Annex W.

<sup>6</sup> Technical dossier/2nd submission/Annex T.

<sup>7</sup> Technical dossier/Additional data/Annex AD\_SI\_NGS.

<sup>8</sup> Technical dossier/2nd submission/Annex T and Additional data March 2019/Annex AD\_SI

<sup>9</sup> Technical dossier/2nd submission/Annex Z.

Although *B. licheniformis* is included in the list of species considered suitable for QPS approach to safety assessment (EFSA BIOHAZ Panel, 2018) [REDACTED]

No issues of concern arising from the genetic modifications were identified by the Panel except the presence of multiple copies of [REDACTED].

### 3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No. 852/2004<sup>10</sup>, with food safety procedures based on hazard analysis and critical control points and in accordance with current good manufacturing practice.<sup>11</sup>

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 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.<sup>12</sup>

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  $\alpha$ -amylase is a single polypeptide of [REDACTED] amino acids.<sup>13</sup> The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be [REDACTED] kDa. The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). A consistent protein pattern was observed across all batches. The gels showed a single major protein band corresponding to an apparent molecular mass of about 55 kDa. No other enzymatic side activities were reported.

The in-house determination of enzyme activity is based on the release of *p*-nitrophenyl by the action of  $\alpha$ -amylase on a *p*-nitrophenyl maltoheptoside substrate with the non-reducing terminal sugar chemically blocked (reaction conditions: pH 5.6, temperature 25°C, reaction time 5 min). The enzyme activity is determined by measuring the release of *p*-nitrophenyl spectrophotometrically at 410 nm. The enzyme activity is quantified relative to an enzyme standard and expressed in  $\alpha$ -amylase units/g (AAU/g).<sup>14</sup> One  $\alpha$ -amylase unit (AAU) is defined as the amount of enzyme required to hydrolyse 10 mg of starch per minute under the conditions of the assay.<sup>15</sup>

The food enzyme has been characterised with regard to its temperature and pH profiles. It has a temperature optimum around 70°C (pH 5.5) and a pH optimum around pH 6.0 (temperature 50°C). 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, the  $\alpha$ -amylase activity decreased rapidly above 60°C, showing no residual activity above 85°C.<sup>16</sup>

#### 3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (Table 1). The average total organic solids (TOS) of the three food enzyme batches for commercialisation was 5.3% (range 4.6–5.9%). The average enzyme activity/TOS ratio of the three food enzyme batches for commercialisation was 672 AAU/mg TOS.

<sup>10</sup> 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.

<sup>11</sup> Technical dossier/1st submission/Annex L.

<sup>12</sup> Technical dossier/Additional data March 2019/Annex AV\_SI.

<sup>13</sup> Technical dossier/1st submission/Annex I.

<sup>14</sup> Technical dossier/1st submission/Annex E.

<sup>15</sup> Technical dossier/Additional data March 2019.

<sup>16</sup> Technical dossier/1st submission/annex J.

**Table 1:** Compositional data of the food enzyme

Parameter	Unit	Batch		
		1	2	3
$\alpha$ -amylase activity	AAU/g batch <sup>(a)</sup>	36,913	33,427	35,778
Protein	%	4.4	3.8	3.4
Ash	%	0.7	0.6	0.4
Water	%	93.4	93.1	95.0
Total organic solids (TOS) <sup>(b)</sup>	%	5.9	5.4	4.6
Activity/mg TOS	AAU/mg TOS	626	619	778

(a): AAU:  $\alpha$ -amylase units (see Section 3.1.3).

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

### 3.3.3. Purity

The lead content in the three commercial batches was below 5 mg/kg which complies with the specification for lead ( $\leq 5$  mg/kg) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006).<sup>17</sup>

The food enzyme complies with the microbiological criteria as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006), which stipulate that *Escherichia coli* and *Salmonella* species are absent in 25 g of sample and total coliforms should not exceed 30 colony forming units per gram. No antimicrobial activity was detected in any of these batches (FAO/WHO, 2006).<sup>18</sup>

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 product was demonstrated in nine independent batches analysed in duplicate. [REDACTED]

The absence of recombinant DNA in the enzyme product was demonstrated by PCR analysis of three batches in triplicate. No DNA was detected [REDACTED]

[REDACTED].<sup>20</sup>

## 3.4. Toxicological data

The food enzyme is intended to be used in distilled alcohol production. In the course of this process, the food enzyme is removed and, consequently, toxicological data were not considered necessary.

### 3.4.1. Allergenicity

The allergenicity assessment considers only the food enzyme and not any carrier or other excipient which may be used in the final formulation.

The allergenicity of  $\alpha$ -amylase produced with the genetically modified *B. licheniformis* strain DP-Dzb44 was assessed by comparing its amino acid sequence 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 the criterion, one match was found. [REDACTED]

No information is available on oral and respiratory sensitisation or elicitation reactions of this  $\alpha$ -amylase.  $\alpha$ -amylase from *A. oryzae* is known as an occupational respiratory allergen associated with baker's asthma (Brisman and Belin, 1991; Sander et al., 1998; Brisman, 2002; Quirce et al., 2002).

<sup>17</sup> Technical dossier/1st submission/annexes G and H.

<sup>18</sup> Technical dossier/1st submission/annex G.

<sup>19</sup> Technical dossier/Additional data March 2019/Annexes AE\_SI, AF\_SI, AJ\_SI-AR\_SI.

<sup>20</sup> Technical dossier/Additional data March 2019/Main response document and Annex AG\_SI.



However, several studies have shown that adults with occupational asthma to a food enzyme (like  $\alpha$ -amylase from *A. oryzae*) may be able to ingest the corresponding enzyme without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Poulsen, 2004; Armentia et al., 2009). Considering the wide use of  $\alpha$ -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  $\alpha$ -amylase in individuals respiratory sensitised to  $\alpha$ -amylase (Losada et al., 1992; Quirce et al., 1992; Baur and Czuppon, 1995; Kanny and Moneret-Vautrin, 1995; Moreno-Ancillo et al., 2004).

According to the information provided, substances or products that may cause allergies or intolerances (Regulation EU 1169/2011)<sup>21</sup> are used as raw materials (██████) in 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.

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 removed, as is the case for distilled alcohol production.

The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions can be excluded.

### 3.5. Dietary exposure

#### 3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in distilled alcohol production at an intended use level of up to 58.1 mg TOS/kg cereal.

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 dextrans to fermentable sugars.

Experimental data have been provided on the removal (> 99%) of protein in the course of distilled alcohol production (Documentation provided to EFSA No 5). The Panel considered the evidence as sufficient to conclude that residual amounts of TOS (including substances other than proteins) are removed by distillation.

#### 3.5.2. Dietary exposure estimation

As residual amounts of the food enzyme are removed by distillation (> 99%), a dietary exposure was not calculated.

## 4. Conclusions

Based on the data provided and including the removal of the food enzyme during the intended food production process, the Panel concluded that the  $\alpha$ -amylase produced with the genetically modified *B. licheniformis* strain DP-Dzb44 does not give rise to safety concerns under the intended conditions of use.

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

## Documentation provided to EFSA

- 1) Dossier "Application for authorisation of  $\alpha$ -amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb44) in accordance with Regulation (EC) No 1331/2008", December 2015. Submitted by Danisco US Inc.

<sup>21</sup> 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.

- 2) Additional information (not full package). August 2018. Submitted by Danisco US Inc.
- 3) Additional information. March 2019. Submitted by Danisco US Inc.
- 4) Summary report on GMM part. March 2018. Delivered by contractor (DTU, Kongens Lyngby, Denmark).
- 5) 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, Dias-Perales A, Castrodeza J, Dueñas-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. <https://doi.org/10.1016/j.aller.2009.05.001>
- Baur X and Czuppon AB, 1995. Allergic reaction after eating  $\alpha$ -amylase (Asp o 2)-containing bred A Case Report. *Allergy*, 50, 85–87. <https://doi.org/10.1111/j.1398-9995.1995.tb02487.x>
- 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 Newman Taylor AJ, 1997. Clinical responses to ingested fungal  $\alpha$ -amylase and hemicellulase in persons sensitized to *Aspergillus fumigatus*? *Allergy*, 52, 346–349. <https://doi.org/10.1111/j.1398-9995.1997.tb01003.x>
- EFSA (European Food Safety Authority), 2009. 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 BIOHAZ Panel (EFSA Panel on Biological Hazards), Ricci A, Allende A, Bolton D, Chemaly M, Davies R, Girones R, Koutsoumanis K, Lindqvist R, Nørrung B, Robertson L, Ru G, Fernández Escámez PS, Sanaa M, Simmons M, Skandamis P, Snary E, Speybroeck N, Ter Kuile B, Threlfall J, Wahlström H, Cocconcelli PS, Peixe L, Maradona MP, Querol A, Suarez JE, Sundh I, Vlák J, Barizzone F, Correia S and Herman L, 2018. Statement on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 7: suitability of taxonomic units notified to EFSA until September 2017. *EFSA Journal* 2018;16(1):5131, 43 pp. <https://doi.org/10.2903/j.efsa.2018.5131>
- EFSA CEF Panel (EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids), 2009. Guidance of EFSA prepared by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. *EFSA Journal* 2009;7(8):1305, 26 pp. <https://doi.org/10.2903/j.efsa.2009.1305>
- 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, Gürtler R, Husøy T, Karenlampi S, Mennes W, Milana MR, Penninks A, Smith A, Tavares Pocas MF, Tlustos C, Wolfle 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), 2011. Scientific Opinion on Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use. *EFSA Journal* 2011;9(6):2193, 54 pp. <https://doi.org/10.2903/j.efsa.2011.2193>
- 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: <http://www.fao.org/3/a-a0675e.pdf>
- Gray GL, Mainzer SE, Rey MW, Lamsa MH, Kindle KL, Carmona C and Requadt C, 1986. Structural genes encoding the thermophilic  $\alpha$ -amylases of *Bacillus stearothermophilus* and *Bacillus licheniformis*. *Journal of Bacteriology*, 166, 635–643.
- Kanny G and Moneret-Vautrin D-A, 1995.  $\alpha$ -amylase contained in bread can induce food allergy. *Journal of Allergy and Clinical Immunology*, 95, 132–133. [https://doi.org/10.1016/S0091-6749\(95\)70161-3](https://doi.org/10.1016/S0091-6749(95)70161-3)
- 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. *Journal of Allergy and Clinical Immunology*, 89, 118–125. [https://doi.org/10.1016/S0091-6749\(05\)80048-X](https://doi.org/10.1016/S0091-6749(05)80048-X)
- Moreno-Ancillo A, Domínguez-Noche C, Gil-Adrados AC and Cosmes PM, 2004. Bread eating induced oral angioedema due to  $\alpha$ -amylase allergy. *Journal of Investigative Allergology and 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. <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 bakers' asthma. *Journal of Allergy and Clinical Immunology*, 90, 970–978.
- Quirce S, Fernandez-Nieto M, Bartolome B, Bombin C, Cuevas M, Sastre J, 2002. Glucoamylase: another fungal enzyme associated with baker's asthma. *AnnAllergy Asthma Immunol*, 89, 197–202.
- 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). *Journal of Allergy and Clinical Immunology*, 102, 256–264.

## Abbreviations

AAU	alpha-amylase units
AMFEP	Association of manufacturers and formulators of enzyme product
CAS	Chemical Abstracts Service
CBS	Westerdijk Fungal Biodiversity Institute
CEF	EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
FAO	Food and Agriculture Organization of the United Nations
GMO	genetically modified organisms
IUBMB	International Union of Biochemistry and Molecular Biology
PCR	polymerase chain reaction
QPS	Qualified presumption of safety
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	total organic solids
WGS	Whole genome sequencing
WHO	World Health Organization