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Safety evaluation of the food enzyme α -amylase from the genetically modified *Bacillus amyloliquefaciens* strain DP-Czb53

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Abstract

The food enzyme α -amylase (4- α -D-glucan glucohydrolase; EC 3.2.1.1) is produced with the genetically modified *B. amyloliquefaciens* strain DP-Czb53 by Danisco US Inc. The genetic modifications do not raise safety concerns, except for the presence of a multicopy plasmid carrying known antimicrobial resistance genes. 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 food enzyme is intended to be used in starch processing for the production of glucose syrups. Toxicological studies and dietary exposure estimation were not considered necessary. 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 by dietary exposure cannot be excluded, but the likelihood for this to occur 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 1332/2008 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 1331/2008² 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:

- it does not pose a safety concern to the health of the consumer at the level of use proposed;
- there is a reasonable technological need;
- 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 Union list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7 (2) of Regulation (EC) No 1332/2008 on food enzymes.

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 Specialities 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 Commission Regulation (EU) No 234/2011 implementing Regulation (EC) No 1331/2008, the Commission has verified that the five applications fall within the scope of the food enzyme Regulation and contains 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.

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* (BD15754), Phospholipase C from a genetically modified strain of *Pichia pastoris* (PRF), 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) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission request to carry out the safety assessment of the food enzyme alpha-amylase from a genetically modified strain of *Bacillus amyloliquefaciens* (DP-Czb53).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier supporting the application for authorisation of the food enzyme α -amylase from a genetically modified *B. amyloliquefaciens* strain DP-Czb53.

Additional information was requested from the applicant during the assessment process on 28 January 2019 and was consequently provided (see 'Documentation provided to EFSA').

Following the reception of additional data by EFSA on 29 January 2020, EFSA requested a clarification teleconference on 12 February 2020, after which the applicant provided additional data on 29 April 2020.

Following the adoption on 17 June 2020, a cross-sectorial issue with an EFSA opinion³ was identified. Subsequently, additional information was requested from the applicant on 19 August 2020, 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, 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 CEP 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 glucohydrolase
Synonyms:	4- α -D-glucan glucanohydrolase
IUBMB No.:	EC 3.2.1.1
CAS No:	9000-90-2
EINECS No:	232-565-6.

α -Amylase catalyses the hydrolysis of 1,4- α -glucosidic linkages in starch (amylose and amylopectin), glycogen and related polysaccharides and oligosaccharides, resulting in the generation of soluble dextrans and other malto-oligosaccharides. The enzyme is intended to be used in starch processing for the production of glucose syrups.

³ Available online: <https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2020.6027>

3.1. Source of the food enzyme

The α -amylase is produced with the genetically modified *Bacillus amyloliquefaciens* strain DP-Czb53, which is deposited in [REDACTED] culture collection [REDACTED] with the deposition number [REDACTED].⁴

B. amyloliquefaciens is included in the list of organisms for which the Qualified Presumption of Safety (QPS) may be applied, provided that the absence of toxigenic activity and the absence of acquired antimicrobial resistance genes is verified for the specific strain used (EFSA BIOHAZ Panel, 2017). The production strain was shown not to be cytotoxic in [REDACTED].⁵

3.1.1. Characteristics of the parental and recipient microorganisms⁶

The recipient strain [REDACTED] is derived from the parental strain [REDACTED].

The production strain is identified as *B. amyloliquefaciens* based on [REDACTED].

3.1.2. Description of introduced sequences⁷

The donor for the α -amylase encoding gene is [REDACTED].

The α -amylase gene, [REDACTED].

3.1.3. Description of genetic modification process⁸

The purpose of the genetic modification was to enable the production strain DP-Czb53 to synthesise α -amylase from [REDACTED].

The antimicrobial susceptibility of *B. amyloliquefaciens* DP-Czb53 was tested [REDACTED].

3.1.4. Safety aspects of the genetic modification⁹

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

The production strain *B. amyloliquefaciens* DP-Czb53 differs from the recipient strain [REDACTED].

The genetic modification does not raise safety concern, except for the presence of a multicopy plasmid carrying known antimicrobial resistance genes.

⁴ Technical dossier/2nd submission/Annex W; Additional data January 2020/Annex AD; Additional data April 2020/Annex AP.

⁵ Technical dossier/Additional data January 2020/Annex AJ.

⁶ Technical dossier/1st submission/Annex Q; Additional data January 2020/Annex AE.

⁷ Technical dossier/1st submission/Annex R; Annex Q; Additional data January 2020/Annex AF.

⁸ Additional data January 2020/Annex AM.

⁹ Technical dossier/2nd submission/Annex V; Additional data January 2020/Annex AF.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004¹⁰, with food safety procedures based on hazard analysis and critical control points, and in accordance with current Good Manufacturing Practice.¹¹

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 and release of the intracellular enzyme, 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.¹² 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.¹³

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

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The α -amylase is a single polypeptide chain of ■ amino acids.¹⁴ The molecular mass of the mature protein, based on the amino acid sequence, was calculated to be ■ kDa. The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis. A consistent protein pattern was observed across all batches. The gel showed a single major protein band corresponding to an apparent molecular mass of about ■ kDa.¹⁵ No other enzymatic side activities were reported.¹⁶

The in-house determination of α -amylase activity is based on hydrolysis of a synthetic substrate *p*-nitrophenyl maltoheptoside with the non-reducing terminal sugar chemically blocked (BPNPG7) in the presence of excess levels of α -glucosidase and amyloglucosidase. The oligosaccharide component of BPNPG7 is attacked by the α -amylase releasing *p*-nitrophenyl maltosaccharide fragments (reaction conditions: pH 7.15, 25°C, 5 min), which are in turn hydrolysed by the other two enzymes to free glucose and *p*-nitrophenol which is determined spectrophotometrically at 410 nm. One Reference Amylase Unit (RAU) is defined as the amount of enzyme required to release 1 micromole of *p*-nitrophenol per minute from BPNPG7 under the conditions described for the assay.¹⁷

The food enzyme has a temperature optimum around 60°C (pH 5.6) and a pH optimum between pH 5.5 and 6.5 (T 50°C). Enzyme activity decreased above 75°C showing almost no residual activity after 8 min at this temperature.¹⁸

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme have been provided for three batches used for commercialisation (Table 1).¹⁹ The average Total Organic Solids (TOS) content of the three commercial enzyme batches was 12.4%. The average enzyme activity/TOS ratio of the three batches for commercialisation is 660.9 RAU/mg TOS.

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/2nd submission/pg. 51-59; Technical dossier/1st submission/Annex K; Additional data April 2020/Annex AR.

¹³ Technical dossier/Additional data January 2020/Annex AL.

¹⁴ Technical dossier/1st submission/Annex H.

¹⁵ Technical dossier/2nd submission/pg. 38.

¹⁶ Technical dossier: pg. 41.

¹⁷ Technical dossier/1st submission/Annex D.

¹⁸ Technical dossier/2nd submission/pg. 42-43.

¹⁹ Technical dossier/2nd submission/pg. 38; Technical dossier/1st submission/Annex F; Additional data April 2020/Annex AQ.

Table 1: Compositional data provided for the food enzyme

Parameter	Unit	Batch		
		1	2	3
α -Amylase activity	RAU/g batch ^(a)	72,861	81,858	85,455
Protein	%	9.69	10.41	8.67
Ash	%	1.06	1.14	1.03
Water	%	87.44	83.74	88.39
Total Organic Solids (TOS) ^(b)	%	11.50	15.12	10.58
α -amylase RAU/mg TOS	Units/mg TOS	633.6	541.4	807.7

(a): RAU: Reference Amylase Unit (see Section 3.3.1).

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

3.3.3. Purity

The lead content in the three commercial batches was below 5 mg/kg²⁰ which complies with the specification for lead (≤ 5 mg/kg)²¹ 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.²² No antimicrobial activity was detected in any of these batches (FAO/WHO 2006).²³

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 strain²⁴ in the food enzyme was demonstrated in [REDACTED]

[REDACTED] No colonies were produced.²⁵

The absence of recombinant DNA in the food enzyme was demonstrated by polymerase chain reaction (PCR) analysis [REDACTED] No DNA was detected [REDACTED]

[REDACTED]²⁶

3.4. Toxicological data

No toxicological tests were provided by the applicant.²⁷ 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 genes 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.

3.4.1. Allergenicity

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

²⁰ LOD: Pb = 0.5 mg/kg.

²¹ Technical dossier/2nd submission/Annex G_updated; Additional data April 2020/Annex AQ.

²² Technical dossier/2nd submission/pg. 40; Technical dossier/1st submission/Annex F; Additional data April 2020/Annex AQ.

²³ Technical dossier/1st submission/Annex F; Technical dossier/2nd submission/Annex G_updated; Additional data April 2020/Annex AQ.

²⁴ Technical dossier/1st submission/Annexes: F, I and G.

²⁵ Technical dossier/1st submission/Annex I; Additional data January 2020/Annexes AG and AH.

²⁶ Technical dossier/Additional data September 2020/Annex AS.

²⁷ Technical dossier/2nd submission/pg. 67.

The potential allergenicity of this α -amylase produced with the genetically modified *B. amyloliquefaciens* DP-Czb53 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 with TAKA-amylase-A, also called Asp o 21 an alpha-amylase from *Aspergillus oryzae*.²⁸

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

α -Amylase from *A. oryzae* (Brisman and Belin, 1991; Sander et al., 1998; Brisman, 2002; Quirce et al., 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 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 this enzyme (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 the genetically modified *B. amyloliquefaciens* DP-Czb53, in individuals sensitised by inhalation to α -amylase cannot be ruled out, but the likelihood of such 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 1169/2011²⁹) are used as raw materials (██████████¹³) 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.

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

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in starch processing for the production of glucose syrups at a recommended use level of up to 45 mg TOS/kg starch.³⁰

In starch processing for glucose syrups production, the food enzyme is added during the liquefaction step where it degrades starch polysaccharides into glucose.

3.5.2. Dietary exposure estimation

The technical information and experimental data provided on the removal of food enzyme TOS during starch processing for glucose syrups production were considered by the Panel as sufficient to exclude this process from the exposure assessment (Annex B in EFSA CEF Panel, 2016). Consequently, a dietary exposure was not calculated.

²⁸ Technical dossier/2nd submission/pg. 68-70; Technical dossier/2nd submission/Annex O.

²⁹ 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.

³⁰ Technical dossier: pg. 61-65.

4. Conclusions

Based on the data provided and the removal of TOS during production of glucose syrups, the Panel concluded that the food enzyme α -amylase produced with the genetically modified *B. amyloliquefaciens* strain DP-Czb53 does not give rise to safety concerns under the intended conditions of use.

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

5. Documentation as provided to EFSA (if appropriate)

- 1) Dossier 'Alpha-amylase from a genetically modified strain of *Bacillus amyloliquefaciens* DP-Czb53'. March 2015. Submitted by Danisco US Inc.
- 2) 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.
- 3) Additional information. January 2020. Submitted by DuPont.
- 4) Additional information. April 2020. Submitted by DuPont.
- 5) Additional information. September 2020. Submitted by DuPont.

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.
- Baur X and Czuppon AB, 1995. Allergic reaction after eating alpha-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 Newman 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), 2009a. 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 (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 BIOHAZ Panel (EFSA Panel on Biological Hazards), Ricci A, Allende A, Bolton D, Chemaly M, Davies R, Girones R, Koutsoumanis K, Herman L, 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, Cocconcilli PS, Klein G (deceased), Peixe L, Maradona MP, Querol A, Suarez JE, Sundh I, Vlák J, Correia S and Fernández Escámez PS, 2017. Statement on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 5: suitability of taxonomic units notified to EFSA until September 2016. *EFSA Journal* 2017;15(3):4663, 20 pp. <https://doi.org/10.2903/j.efsa.2017.4663>
- EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 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> and Annex B – Process-specific technical data used in exposure assessment of food enzymes. Available online: https://efsa.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.2903%2Fj.efsa.2016.4581&file=efs24581-sup-0001-Annex_B.pdf
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), 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: <http://www.fao.org/3/a-a0675e.pdf>

- Kanny G and Moneret-Vautrin DA, 1995. Alpha-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. *Journal of Allergy and Clinical Immunology*, 89(1 Pt 1), 118–125.
- 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 and 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. *Journal of Allergy and Clinical Immunology*, 90(6 Pt 1), 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 Immunology*, 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

bp	base pair
BPNG7	blocked <i>p</i> -nitrophenyl maltoheptoside
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
CHO	Chinese hamster ovary
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
MIC	minimum inhibitory concentration
PCR	polymerase chain reaction
RAU	Reference amylase unit
RNA	ribonucleic acid
QPS	Qualified presumption of safety
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TOS	Total Organic Solids
WHO	World Health Organization