SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 4 December 2018

doi: 10.2903/j.efsa.2019.5549

Safety evaluation of the food enzyme α-amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb54)

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), Vittorio Silano, José Manuel Barat Baviera, Claudia Bolognesi, Beat Johannes Brüschweiler, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Evgenia Lampi, Alicja Mortensen, Gilles Rivière, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis, Holger Zorn, Lieve Herman, Jaime Aguilera, Magdalena Andryszkiewicz, Zoltán Diveki and Andrew Chesson

Abstract

The food enzyme α-amylase (4-α-D-glucan glucanohydrolase; EC 3.2.1.1) is produced with the genetically modified *Bacillus licheniformis* strain DP-Dzb54 by Danisco. The α-amylase is intended to be used in starch processing for the production of glucose syrups. Residual amounts of total organic solids are removed by the purification steps applied during the production of glucose syrups; consequently, dietary exposure was not calculated. The parental strain meets all the requirements for the Qualified Presumption of Safety approach for risk assessment, except the absence of acquired antimicrobial resistance genes. However, this has no practical consequence for the food enzyme as it has been shown not to contain viable cells and DNA from the production strain. 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 and no 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 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.

Keywords: food enzyme, α-amylase, 1,4-α-D-glucan glucanohydrolase, EC 3.2.1.1, *Bacillus licheniformis*, DP-Dzb54, genetically modified microorganism

Requestor: European Commission

Question number: EFSA-Q-2015-00666

Correspondence: fip@efsa.europa.eu

Panel members: José Manuel Barat Baviera, Claudia Bolognesi, Beat Johannes Brüschweiler, Andrew Chesson, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Evgenia Lampi, Alicja Mortensen, Gilles Rivière, Vittorio Silano, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis, Holger Zorn.

Note: The full opinion will be published in accordance with Article 12 of Regulation (EC) No 1331/2008 once the decision on confidentiality will be received from the European Commission.

Acknowledgements: The CEP Panel wishes to thank the Working Group on Food Enzymes of the former EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) for the preparatory work on this scientific output. The CEP Panel wishes to acknowledge all European competent institutions, Member State bodies and other organisations that provided data for this scientific output.

Suggested citation: 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, Herman L, Aguilera J, Andryszkiewicz M, Diveki Z and Chesson A, 2019. Scientific Opinion on the safety evaluation of the food enzyme α-amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb54). EFSA Journal 2019;17(1):5549, 11 pp. https://doi.org/10.2903/j.efsa.2019.5549

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

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 4
1.2. Information on existing authorisation and evaluations 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 5
3.1.2. Characteristics of the inserted 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 6
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
3.5. Allergenicity 8
3.6. Dietary exposure 9
3.6.1. Intended use of the food enzyme 9
3.6.2. Dietary exposure estimation 9
4. Conclusions 9
Documentation provided to EFSA 9
References 10
Abbreviations 11

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 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 approval via an EU Community list.

The 'Guidance on submission of a dossier on food enzymes for safety evaluation' (EFSA CEF Panel, 2009) lays down the administrative, technical and toxicological data required.

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

1.1.1. Background as provided by the European Commission

An application has been introduced by the company Danisco for the authorisation of the food enzyme α-amylase obtained from a genetically modified *Bacillus licheniformis* strain (DP-Dbz54).

Following the requirements of Article 12.1 of Commission Regulation (EU) No 234/2011[3] implementing Regulation (EC) No 1331/2008[4], the Commission has verified that the application falls within the scope of the food enzyme Regulation and contains all the elements required under Chapter II of that Regulation.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessment on the food enzyme α-amylase obtained from a genetically modified *Bacillus licheniformis* (strain DP-Dbz54) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

[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.

[4] 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.

1.2. Information on existing authorisation and evaluations

The applicant reports that the Australian/New Zealand, Danish, French, Canadian and Brazilian authorities have authorised the use of α-amylase from genetically modified *B. licheniformis* in a number of food and beverage manufacturing processes.

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-Dzb54).

Additional information was sought from the applicant during the assessment process in response to requests from EFSA sent on 13 July 2017 and on 27 June 2018 and was subsequently provided (see 'Documentation provided to EFSA').

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA 'Guidance on transparency in the scientific aspects of risk assessment' (EFSA, 2009) 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's of the 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:	α-amylase
Systematic name:	4-α-D-glucan glucanohydrolase
Synonyms:	Glycogenase, endoamylase, 1,4-α-D-glucan glucanohydrolase
IUBMB No:	EC 3.2.1.1
CAS No:	9000-90-2
EINECS No:	232-565-6

The 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 dextrins and other malto-oligosaccharides. It is intended to be used in starch processing for modified starch and glucose syrup production to be used in solid foods and beverages.

3.1. Source of the food enzyme

The α-amylase is produced with a genetically modified strain of *B. licheniformis* (DP-Dzb54), which is deposited in the CBS International Culture Collection (the Netherlands) with deposition umber [redacted][5]

3.1.1. Characteristics of the parental and recipient microorganisms

The parental microorganism is the *B. licheniformis* strain [redacted]. *B. licheniformis* is recommended for the Qualified Presumption of Safety (QPS) status, with the qualification that the absence of acquired antimicrobial resistance genes and toxigenic activity are verified for the specific strain used (EFSA BIOHAZ Panel, 2017, 2018). The absence of cytotoxicity activity was confirmed for the parental strain [redacted] on CHO_K1 (Chinese hamster ovary cells).[6] The parental strain was taxonomically identified as *B. licheniformis* by [redacted] However, the strain

[5] Technical dossier/Additional information April 2018/Annex X.
[6] Technical dossier/Additional information April 2018/Annex E.
[7] Technical dossier/1st submission/Annex K.

was not tested for other antimicrobial resistances. Therefore, the parental strain cannot be considered to qualify for the QPS status.

The recipient strain *B. licheniformis* [redacted] was developed from the parental strain [redacted][8][redacted][9][redacted]

3.1.2. Characteristics of the inserted sequences[8]

[redacted]

3.1.3. Description of the genetic modification process[8]

[redacted][10]

3.1.4. Safety aspects of the genetic modification

The production *B. licheniformis* strain DP-Dzb54 differs from the parental strain [redacted]

Genotypic stability of the *B. licheniformis* DP-Dzb54 production strain was demonstrated [redacted][11] The consistency of enzyme activity observed in three batches intended for commercialisation (Table 1) indicates that the production strain is phenotypically stable.

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 (HACCP), and in accordance with current Good Manufacturing Practice (GMP).[13]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, fed-batch fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration leaving a supernatant containing the food enzyme. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in which enzyme protein is retained while most of

[8] Technical dossier/2nd submission/Annex U.
[9] Technical dossier/Additional information April 2018/Annex F.
[10] Technical dossier/Additional information September 2018/Annex AA.
[11] Technical dossier/2nd submission/Annex AB.
[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.
[13] Technical dossier/1st submission/Annex M.

the low molecular weight material passes the filtration membrane and is discarded.[14] 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.[15]

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 subject of this assessment is a single polypeptide chain of [redacted] amino acids.[16] The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be about [redacted] kDa. The protein pattern of the food enzyme was determined using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis for three batches. A single main protein band was detected for all three batches, and its apparent molecular mass based on this technique is about 55 kDa. No other enzymatic side activities were reported.[17]

The α-amylase activity is quantified based on the hydrolysis of non-reducing-end blocked *p*-nitrophenyl maltoheptaoside substrate combined with excess levels of α-glucosidase and glucoamylase and is expressed in Thermostable α-Amylase Units/g (TAU/g). The analytical principle is based on the reaction between *p*-nitrophenyl maltosaccharide fragments (formed from the reaction between the substrate and the α-amylase; reaction conditions: pH 7.15, 25°C) and the α-glucosidase, which liberates *p*-nitrophenol, which is determined by spectrophotometry at 410 nm. Activity is calculated based on a standard sample with a known enzymatic activity value.[18]

The α-amylase has been characterised regarding its activity depending on temperature and pH. It has a temperature optimum around 70°C (pH 5.8) and a pH optimum around pH 4.5 (50°C).[19] Thermostability was tested [redacted][20]

3.3.2. Chemical parameters

Data on the composition of the food enzyme have been provided for three commercial batches[21] (Table 1) of the concentrate before addition of any additives or other standardisation or stabilisation ingredients. The average total organic solids (TOS) was 13.26% (w/w); the values ranged from 12.69% to 14.19% (Table 1). The TOS content is a calculated value derived as 100% minus % water minus % ash. The average enzyme activity/TOS ratio of the food enzyme, expressed as Thermostable α-Amylase Units (TAU) (see Section 3.1.3), was 250 TAU/mg TOS.

Table 1: Compositional data of the food enzyme

Parameter	Unit	Batches		
		1	2	3
α-Amylase activity	TAU/g batch[(a)]	33,033	33,984	32,405
Protein	% (w/w)	11.08	11.20	10.6
Ash	% (w/w)	0.94	0.83	0.74
Water	% (w/w)	86.15	84.98	86.57
Total organic solids (TOS)[(b)]	% (w/w)	12.91	14.19	12.69
α-amylase activity/mg TOS	TAU/mg TOS	256	239	255

(a): TAU/g batch: Thermostable α-Amylase Units (see Section 3.3.1).
(b): TOS calculated as 100% – % water – % ash.

[14] Technical dossier/1st submission/Annex N.
[15] Technical dossier/Additional information April 2018/Annex I.
[16] Technical dossier/1st submission/Annex I.
[17] Technical dossier/2nd submission/Updated dossier/Section 3.2.1.1.2.1.
[18] Technical dossier/1st submission/Annex E.
[19] Technical dossier/2nd submission/Updated dossier/Section 3.2.1.1.2.3.
[20] Technical dossier/Additional information April 2018/Annex D.
[21] Technical dossier/Additional information April 2018/Annex A–C.

3.3.3. Purity

The applicant provided data on three batches of the food enzyme demonstrating that the content of lead (≤ 5 mg/kg) was below the specification levels set for food additives (FAO/WHO, 2006). No antimicrobial activity was detected in any of these batches according to the levels specified in (FAO/WHO, 2006).[22]

The food enzyme complies with the microbiological criteria as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006), which stipulate that *E. coli* and *Salmonella* species are absent in 25 g of sample and total coliforms are not more than 30 colony forming units (CFU) per gram.[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 production strain could not be detected in a test volume of 1.2 mL of nine batches of the liquid final enzyme product,[24] by culturing on non-selective agar and incubating for 48 h at 36°C.[25]

The absence of recombinant DNA in the food enzyme was demonstrated [redacted][26]

3.4. Toxicological data

B. licheniformis is included in the list of species considered potentially suitable for the QPS approach provided that the absence of acquired antimicrobial resistance genes and toxigenic activity are verified for the specific strain used (EFSA BIOHAZ Panel, 2017, 2018). The parental strain *B. licheniformis* strain [redacted] has been unequivocally identified and has been shown to be non-cytotoxic, but the strain has not been tested for antimicrobial susceptibility (other than for chloramphenicol). Although all other requirements for the QPS have been met, in the absence of such data, the parental strain cannot be considered as suitable for the QPS approach.

The conclusion on the QPS status of the parental strain also applies to the production strain. However, no practical consequence for the food enzyme is expected from the possible presence of unrecognised 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.

3.5. Allergenicity

The potential allergenicity of α-amylase produced with the genetically modified *B. licheniformis* strain DP-Dzb54 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 window of 80 amino acids as the criterion, no match was found.[27]

Taking into account the wide use of α-amylase as a food additive only a low number of case reports have been described in literature focussed on allergic reactions upon oral exposure to α-amylase in individuals respiratory sensitised to α-amylase (Baur and Czuppon, 1995; Kanny and Moneret-Vautrin, 1995; Moreno-Ancillo et al., 2004). Other studies (e.g. Cullinan et al., 1997; Poulsen, 2004; Armentia et al., 2009) showed that clinical reactions upon oral exposure to α-amylase did not occur in α-amylase sensitised individuals.

[22] Technical dossier/1st submission/Annex G.
[23] Technical dossier/1st submission/Annex G and Additional information April 2018/Annex I.
[24] Technical dossier/Additional information April 2018/Annex G.
[25] Technical dossier/2nd submission/Annex H.
[26] Technical dossier/Additional information September 2018/Annex AC.
[27] Technical dossier/1st submission/Annex T.

According to the information provided, substances or products that may cause allergies or intolerances (Regulation (EU) No 1169/2011[28]) are used as raw materials (soybean meal) 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/fungal 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. 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 α-amylase produced with the genetically modified *B. licheniformis* strain DP-Dzb54 cannot be excluded, but the likelihood of such reactions occurring is considered to be low.

3.6. Dietary exposure

3.6.1. Intended use of the food enzyme

The food enzyme is intended to be used in the starch processing for the production of glucose syrups, at the intended use level of up to 26 mg TOS/kg liquefied starch.

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

Experimental data have been provided on the removal (> 99%) of protein in the course of starch processing for the production of glucose syrups (Documentation provided to EFSA No 6). 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.

3.6.2. Dietary exposure estimation

As residual amounts of TOS are removed by the purification steps applied during the production of glucose syrups (by > 99%), a dietary exposure was not calculated.

4. Conclusions

Based on the data provided, the Panel concluded that the food enzyme amylase produced with the genetically modified *B. licheniformis* strain DP-Dzb54 does not give rise to safety concerns under the intended conditions of use.

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

Documentation provided to EFSA

1) Application for authorisation of A-amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb54) in accordance with Regulation (EC) No 1331/2008. February 2015. Submitted by Danisco US Inc.
2) Additional information. April 2018. Submitted by Danisco US Inc.
3) Additional information. September 2018. Submitted by Danisco US Inc.
4) Summary report on GMM part for α-amylase produced by *Bacillus licheniformis* strain DP-Dzb54, EFSA-Q-2015-00666. 2015. Delivered by the Technical University of Denmark.

[28] 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.

5) Summary report on technical data and dietary exposure related to α-amylase from a strain of *Bacillus licheniformis* (strain DP-Dzb54) by Danisco US Inc. October 2016. Delivered by Hylobates Consulting and BiCT.
6) Additional information on '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.

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), 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/doi: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, 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 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, Vlak 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 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 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 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, vanLoveren H, Selb R and FernandezDumont 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 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.

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.

Abbreviations

CAS	Chemical Abstracts Service
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
GMM	genetically modified microorganism
GMP	Good Manufacturing Practices
HACCP	Hazard Analysis and Critical Control Points
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
TAU	Thermostable α-Amylase Units
TOS	total organic solids
WGS	whole genome sequence
WHO	World Health Organization