SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 25 November 2021

doi: 10.2903/j.efsa.2022.7004

Safety evaluation of the food enzyme cyclomaltodextrin glucanotransferase from *Anoxybacillus caldiproteolyticus* strain St-88

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), Claude Lambré, José Manuel Barat Baviera, Claudia Bolognesi, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis, Holger Zorn, Boet Glandorf, Lieve Herman, Jaime Aguilera, Yi Liu and Andrew Chesson

Abstract

The food enzyme cyclomaltodextrin glucanotransferase ((1→4)-α-D-glucan 4-α-D-[(1→4)-α-D-glucano]-transferase (cyclising), EC 2.4.1.19) is produced with *Anoxybacillus caldiproteolyticus* strain St-88 by PureCircle USA. It is intended to be used in the manufacture of glycosylated steviol glycosides. Residual amounts of total organic solids are removed by the purification steps applied during the production of the modified steviol glycosides; consequently, dietary exposure was not calculated. For the same reason, toxicological studies other than assessment of allergenicity were not considered necessary. Similarity of the amino acid sequence of the food enzyme to those of known allergens was searched and four matches were 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 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, cyclomaltodextrin glucanotransferase, cyclodextrin glycosyltransferase, (1→4)-α-D-glucan:(1→4)-α-D-glucan 4-α-D-[(1→4)-α-D-glucano]-transferase, EC 2.4.1.19, *Anoxybacillus caldiproteolyticus*

Requestor: European Commission

Question number: EFSA-Q-2015-00230

Correspondence: FIP@efsa.europa.eu

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

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.

Declarations of interest: The declarations of interest of all scientific experts active in EFSA's work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

Acknowledgments: The 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), Lambré C, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mengelers M, Mortensen A, Rivière G, Steffensen I-L, Tlustos C, Van Loveren H, Vernis L, Zorn H, Glandorf B, Herman L, Aguilera J, Liu Y and Chesson A, 2022. Scientific Opinion on the safety evaluation of the food enzyme cyclomaltodextrin glucanotransferase from *Anoxybacillus caldiproteolyticus* strain St-88. EFSA Journal 2022;20(1):7004, 10 pp. https://doi.org/10.2903/j.efsa.2022.7004

ISSN: 1831-4732

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

Table of contents

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:

- 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 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, 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.

Six applications have been introduced by the companies "Decernis, LLC", "Keller and Heckman LLP", the "Association of Manufacturers and Formulators of Enzyme Products (AMFEP)" and "Novozymes A/S" for the authorisation of the food enzymes Cyclomaltodextrin glucanotransferase from *Geobacillus stearothermophilus*, Dextranase from *Chaetomium gracile*, Subtilisin from *Bacillus licheniformis*, Mucorpepsin from *Rhizomucor miehei*, Animal rennet consisting of chymosin and pepsin from the abomasum of *Bos primigenius* (cattle), *Bubalus bubalis* (buffalo), *Capra aegagrus hircus* (goat) and *Ovis aries* (sheep), and Lipase from a genetically modified strain of *Aspergillus niger* (strain NZYM-DB) respectively.

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 six 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, 11.03.2011, pp. 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 Cyclomaltodextrin glucanotransferase from *Geobacillus stearothermophilus*, Dextranase from *Chaetomium gracile*, Subtilisin from *Bacillus licheniformis*, Mucorpepsin from *Rhizomucor miehei*, Animal rennet consisting of chymosin and pepsin from the abomasum of *Bos primigenius* (cattle), *Bubalus bubalis* (buffalo), *Capra aegagrus hircus* (goat) and *Ovis aries* (sheep), and Lipase from a genetically modified strain of *Aspergillus niger* (strain NZYM-DB) 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 the food enzyme cyclomaltodextrin glucanotransferase from *Geobacillus stearothermophilus*. Recent data identified the production microorganism as *Anoxybacillus caldiproteolyticus* (Section 3.1). Therefore, this name will be used in this opinion instead of *G. stearothermophilus*.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme cyclomaltodextrin glucanotransferase from *Geobacillus stearothermophilus*.

Additional information was requested from the applicant during the assessment process on 18 May 2020 and 29 April 2021 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) and following the relevant existing guidance documents of EFSA Scientific Committee.

The current Guidance on the submission of a dossier on food enzymes for safety evaluation (EFSA, 2009a) as well as the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) has been followed for the evaluation of the application.

3. Assessment

IUBMB nomenclature	cyclomaltodextrin glucanotransferase
Systematic name	(1→4)-α-D-glucan 4-α-D-[(1→4)-α-D-glucano]-transferase (cyclising)
Synonyms	cyclodextrin glycosyltransferase, α-cyclodextrin glucanotransferase
IUBMB No.	EC 2.4.1.19
CAS No.	9030-09-5
EINECS No.	618-522-8

Cyclomaltodextrin glucanotransferase catalyses the transglycosylation of glucans by formation of a (1→4)-α-D-glucosidic bond, resulting in the generation of mainly cyclodextrins and transglycosylated glucans. It is intended to be used in the manufacture of glycosidated steviol glycosides.

3.1. Source of the food enzyme

The cyclomaltodextrin glucanotransferase is produced with the non-genetically modified *Anoxybacillus caldiproteolyticus* strain St-88, which is deposited at the German Collection of Microorganisms and Cell Cultures (DSMZ, Germany), with deposit number [redacted].[4] The production strain is a wild-type isolated from soil and was identified as *A. caldiproteolyticus* by 16S rRNA, *Spo0A*, *RecA* and *RecN* gene sequence analysis.[5]

[4] Technical dossier/Additional information March 2021/Attachment A.
[5] Technical dossier/Additional information March 2021/Attachment B.

A. caldiproteolyticus St-88 was not found to be cytotoxic in VERO cells.[6] Whole genome sequence (WGS) analysis of the strain did not indicate the presence of virulence associated genes, nor the presence of acquired genes involved in antimicrobial resistance.[7]

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[8,9], with food safety procedures based on hazard analysis and critical control points, and in accordance with current good manufacturing practice.

The production strain is grown as a pure culture, using a typical industrial medium in a submerged fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration. 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 mass material passes the filtration membrane and is discarded.[10] The applicant provided information on the identity of the substances used to control the fermentation and the subsequent downstream processing of the food enzyme.[11]

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 cyclomaltodextrin glucanotransferase is a single polypeptide chain of 711 amino acids.[12] The molecular mass, derived from the amino acid sequence, was calculated to be 78.9 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 protein band corresponding to an apparent molecular mass of about 70 kDa, consistent with the expected mass of the enzyme. The protein profile also included several bands of different staining intensity. No other enzymatic activities were reported.

The in-house determination of cyclomaltodextrin glucanotransferase activity is based on the partial hydrolysis of starch (reaction conditions: pH 5.2, 40°C, 20 min). After adding an aliquot of the enzyme/starch solution to an iodine solution (3 min, 25°C), the absorbance is monitored spectrophotometrically at 660 nm. The enzyme activity is expressed in cyclomaltodextrin glucanotransferase units (U)/g. One unit is defined as the amount of enzyme capable of reducing the light transmission to 50% in 10 min under the conditions of the assay.[13]

The food enzyme has a temperature optimum at around 60°C (pH 7.0) and a pH optimum between pH 6.0 and 7.0 (60°C). Thermostability was tested after a pre-incubation of the food enzyme for 120 min at different temperatures. Under the conditions (pH 7.0) of the applied temperature stability assay, cyclomaltodextrin glucanotransferase shows around 40% residual activity at 80°C.[14]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (Table 1).[15] The mean total organic solids (TOS) was 1.6% and the mean enzyme activity/TOS ratio about 0.1.

[6] Technical dossier/Additional information March 2021/Attachment C.
[7] Technical dossier/Additional information March 2021/Attachment B and Additional information September 2021.
[8] 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.
[9] Technical dossier/Additional information March 2021/Attachment D.
[10] Technical dossier/2nd submission/p. 19 and Annex 9.
[11] Technical dossier/Additional information March 2021.
[12] Technical dossier/2nd submission/Annex 13.
[13] Technical dossier/2nd submission/Annex 2B.
[14] Technical dossier/p. 13–15.
[15] Technical dossier/Additional information March 2021/Attachment G.

Table 1: Compositional data of the food enzyme

Parameters	Unit	1	2	3
Cyclomaltodextrin glucanotransferase activity	U/mL batch[(a)]	2.4	2.2	1.8
Protein	%	NA[(b)]	NA	NA
Ash	%	2.1	2.1	1.7
Water	%	96.4	96.1	96.7
Total organic solids (TOS)[(c)]	%	1.5	1.8	1.6
Activity/mg TOS	U/mL TOS	0.2	0.1	0.1

(a): U: cyclodextrin glucanotransferase units (see Section 3.3.1).
(b): NA: not analysed.
(c): TOS calculated as 100% – % water – % ash.

3.3.3. Purity

The lead content in the three commercial batches tested was below 0.019 mg/kg, 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).[16,17]

The food enzyme complies with the microbiological criteria (for total coliforms, *Escherichia coli* and *Salmonella*) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006). No antimicrobial activity was detected in any of the three tested batches (FAO/WHO, 2006).[16]

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

3.3.4. Viable cells of the production strain

The absence of viable cells of the production strain in the food enzyme at the end of the killing was demonstrated in three independent batches analysed in triplicate. Ten mL of product were diluted in 990 mL of 0.9% NaCl. From this, 100 aliquots of 1 mL were plated on non-selective medium and incubated at 55°C for 2 days. A positive control was provided. No colonies of the production strain were observed.[18]

3.4. Toxicological data

No toxicological tests were provided by the applicant. The food enzyme is intended to be used in the production of modified steviol glycosides. In the course of this process, the food enzyme is removed by the applied purification steps (see Section 3.5) and, consequently, no toxicological studies other than assessment of allergenicity are needed for the assessment of this food enzyme.

3.4.1. Allergenicity

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

The potential allergenicity of the cyclomaltodextrin glucanotransferase produced with the non-genetically modified *A. caldiproteolyticus* strain St-88 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 sliding window of 80 amino acids as the criterion, four matches were found. The matching allergen(s) were α-amylase A type-1/2 from *Aspergillus oryzae*, α-amylase A type 3 precursor from *A. oryzae*, glucoamylase from *Schizophyllum commune* (split gill fungus) and a putative maltase from *Aedes aegypti* (yellow fever mosquito).[19]

Both glucoamylase from *S. commune* (Toyotome et al., 2014) and α-amylase from *A. oryzae* (Brisman and Belin, 1991; Sander et al., 1998; Brisman, 2002; Quirce et al., 2002) are known as occupational respiratory allergens associated with baker's asthma. However, several studies have

[16] Technical dossier/2nd submission/p. 13 and annexes 14–18.
[17] LoD:Pb = 1 mg/kg.
[18] Technical dossier/ Additional information September 2021/Attachment K.
[19] Technical dossier/Additional information March 2021/Attachment J.

shown that adults with occupational asthma caused by an 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 has been described in the literature that focused on allergic reactions upon oral exposure to α-amylase in individuals respiratorily-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). Such information has not been reported for glucoamylase. The maltase from the yellow fever mosquito is associated with bites, but no effects of oral exposure to this enzyme have been reported.

No information is available on oral and respiratory sensitisation or elicitation reactions of this cyclomaltodextrin glucanotransferase. The applicant conducted a literature search looking for possible adverse reactions upon consumption of glucanotransferases and no record was found.[11]

According to the information provided, substances or products that may cause allergies or intolerances (corn steep liquor) are used as raw materials 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 materials employed as protein sources are not expected to be present in the food enzyme.

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 to occur is considered to be low.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The cyclomaltodextrin glucanotransferase is intended to be used in the manufacture of modified steviol glycosides, at a recommended use level of up to 6.9 g TOS/kg raw material.[20]

A flowchart depicting the manufacturing process steps of the modified steviol glycosides has been provided.[20] The food enzyme is added to a mixture of liquefied starch and steviol glycosides. The starch is from tapioca and the steviol glycosides are extracted from the *Stevia* plant. The cyclomaltodextrin glucanotransferase transfers glucose units from starch to the steviol glycosides to produce the glucosylated steviol glycosides. [redacted].

The Panel considered that the efficiency of the described purification steps in the production of the modified steviol glycosides is essentially the same as those in the production of glucose syrups. Based on data provided on thermostability (see Section 3.3.1), it is expected that the enzyme is inactivated during the manufacturing processes of the glycosylated steviol glycoside. Cyclomaltodextrin glucanotransferase activity was not detected in two samples of the modified steviol glycoside powder.[21] No protein was detected in the glucosylated steviol glycosides powder by a copper-based colorimetric assay in six samples.[22]

3.5.2. Dietary exposure estimation

The technical information and experimental data provided on the removal of food enzyme TOS during the manufacturing of the modified steviol glycosides were considered by the Panel as sufficient to exclude this process from the exposure estimation. Consequently, a dietary exposure was not calculated.

Since a toxicological assessment and the calculation of dietary exposure were considered unnecessary by the Panel, the margin of exposure was not calculated.

[20] Additional data March 2021.
[21] Technical dossier/Annex 10.
[22] Additional data March 2021/Attachment I, LoD = 5 μg/mL.

4. Conclusions

Based on the data provided and the removal of TOS during the intended food process, the Panel concluded that the food enzyme cyclomaltodextrin glucanotransferase produced with *A. caldiproteolyticus* strain St-88 does not give rise to safety concerns under the intended conditions of use.

5. Documentation as provided to EFSA

1) Cyclomaltodextrin glucanotransferase derived from *Geobacillus stearothermophilus*. April 2015. Submitted by PureCircle USA.
2) Additional information. March 2021. Submitted by PureCircle USA.
3) Additional information. September 2021. Submitted by PureCircle USA.

References

Armentia A, Dias-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 bred. A case report. Allergy, 50, 85–87.

Brisman J, 2002. Baker's asthma. Occupational and Environmental Medicine, 59, 498–502.

Brisman J and Belin L, 1991. Clinical and immunological responses to occupational exposure to α-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 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: https://www.fao.org/3/a-a0675e.pdf

Kanny G and Moneret-Vautrin D-A, 1995. A-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 α-amylase inhalation: clinical and immunologic findings and bronchial response patterns. 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 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.

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 and Sastre J, 2002. Glucoamylase: another fungal enzyme associated with baker's asthma. Annals of Allergy, 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.

Toyotome T, Satoh M, Yahiro M, Watanabe A, Nomura F and Kamei K, 2014. Glucoamylase is a major allergen of *Schizophyllum commune*. Clinical and Experimental Allergy, 44, 450–457.

Abbreviations

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
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GM	genetically modified
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
LoD	limit of detection
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TOS	total organic solids
WHO	World Health Organization