SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 7 July 2022

doi: 10.2903/j.efsa.2022.7463

Safety evaluation of the food enzyme α-amylase from *Cellulosimicrobium funkei* strain AE-AMT

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Abstract

The food enzyme α-amylase (4-α-D-glucan glucanohydrolase; EC 3.2.1.1) is produced with the non-genetically modified *Cellulosimicrobium funkei* strain AE-AMT by Amano Enzyme Inc. The food enzyme is free from viable cells of the production organism. It is intended to be used in starch processing for maltotriose production. Since residual amounts of total organic solids (TOS) are removed by purification steps applied during starch processing, the estimation of a dietary exposure is considered unnecessary. Genotoxicity tests did not indicate a safety concern. The systemic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rats. The Panel identified a no observed adverse effect level at the highest dose of 230 mg TOS/kg body weight (bw) per day. Similarity of the amino acid sequence of the food enzyme to those of known allergens was searched and nine 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 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, EC 3.2.1.1, 4-α-D-glucan glucanhydrolase, *Microbacterium imperiale*, *Cellulosimicrobium funkei*, maltotriose

Requestor: European Commission

Question number: EFSA-Q-2014-00544

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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: If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

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, Andryszkiewicz M, Gomes A, Liu Y, Rainieri S and Chesson A, 2022. Scientific Opinion on the safety evaluation of the food enzyme α-amylase from *Cellulosimicrobium funkei* strain AE-AMT. EFSA Journal 2022;20(8):7463, 12 pp. https://doi.org/10.2903/j.efsa.2022.7463

ISSN: 1831-4732

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

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1. Introduction

Article 3 of the Regulation (EC) No 1332/2008[1] provides definition for 'food enzyme' and 'food enzyme preparation'.

'Food enzyme' means a product obtained from plants, animals or micro-organisms or products thereof including a product obtained by a fermentation process using micro-organisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.

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

Before January 2009, food enzymes other than those used as food additives were not regulated or were regulated as processing aids under the legislation of the Member States. On 20 January 2009, Regulation (EC) No 1332/2008 on food enzymes came into force. This Regulation applies to enzymes that are added to food to perform a technological function in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food, including enzymes used as processing aids. Regulation (EC) No 1331/2008[2] established the European Union (EU) procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

- 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 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.

Two applications have been introduced by the company Amano Enzyme Inc. for the authorisation of the food enzymes Alpha-amylase from *Microbacterium imperial* strain AE-AMT and Triacylglycerol lipase from *Penicillium roqueforti* strain AE-LRF.

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 applications fall within the scope of the food enzyme Regulation and contain 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 enzymes Alpha-amylase from *Microbacterium imperial* strain AE-AMT and Triacylglycerol lipase form *Penicillium roqueforti* strain AE-LRF 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, 11.03.2011, pp. 15–24.

1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of food enzyme α-amylase from *M. imperial* strain AE-AMT.

Recent data identified the production microorganism as *Cellulosimicrobium funkei* (Section 3.1). Therefore, this name will be used in this opinion instead of *Microbacterium imperial*.

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 from *M. imperiale* strain AE-AMT. The dossier was updated on 7 July 2014.

Additional information was requested from the applicant during the assessment process on 28 May 2015 and 21 July 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) and following the relevant guidance documents of the 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) have been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance with the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021a).

3. Assessment

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

α-Amylases catalyse 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. The food enzyme is intended to be used in starch processing for maltotriose production.

3.1. Source of the food enzyme

The α-amylase is produced with the non-genetically modified bacterium *C. funkei* strain AE-AMT, which is deposited at [redacted] with deposit number [redacted][4]

The production strain was identified as *C. funkei* [redacted][5][6]

[4] Technical dossier/Additional data February 2022/Annex 1.
[5] Technical dossier/Additional data February 2022/Annex 2.
[6] Technical dossier/Additional data February 2022/Annex 5.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[7], with food safety procedures based on hazard analysis and critical control points, and in accordance with current good manufacturing practice.[8]

The production strain is grown as a pure culture using a typical industrial medium in a submerged 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 filtrate 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 mass material passes the filtration membrane and is discarded. The enzyme concentrate is formulated into a final dry or liquid enzyme preparation.[9] The applicant provided information on the identity of the substances used to control the fermentation and in the subsequent downstream processing of the food enzyme.[10]

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

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The α-amylase is a single polypeptide chain of [redacted] amino acids.[11] The molecular mass of the mature protein, calculated from the amino acid sequence, is [redacted] kDa.[11] Three food enzyme batches were analysed by gel chromatography and showed a similar protein pattern.[12] No other enzymatic activities were reported.[13]

The in-house determination of α-amylase activity is based on hydrolysis of starch (reaction conditions: pH 6.0, 40°C, 15 min). The enzymatic activity is determined by measuring the release of reducing sugar by a colorimetric assay spectrophotometrically at 520 nm.[14] The enzyme activity is expressed in Units/g. One Unit is defined as the quantity of enzyme producing an amount of reducing sugars equivalent to 1 μmol of glucose per minutes under the conditions of the assay.[15]

The food enzyme has a temperature optimum between 45°C and 55°C (pH 7.0 and a pH optimum between pH 5.0 and 8.0 (40°C). Thermostability was tested after a pre-incubation of the food enzyme for 10 min at different temperatures (pH 7.0). The enzyme activity decreased above 40°C, showing no residual activity above 55°C.[16]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation and one batch produced for the toxicological tests (Table 1).[17] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation is 6.2% and the mean enzyme activity/TOS ratio is 27.0 U/mg TOS. Prior to drying the food enzyme, it is stabilised with [redacted] (~ 34%).

[7] 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, p. 3–21.
[8] Technical dossier/Annex 5.
[9] Technical dossier/p. 29-36/Annex 6.
[10] Technical dossier/p. 30/Annex 7/Additional data July 2015.
[11] Technical dossier/Additional data February 2022/Annex 3.
[12] Technical dossier/p. 23.
[13] Technical dossier/p. 24–25.
[14] Technical dossier/Annex 3.
[15] Technical dossier/p. 24/Annex 3.
[16] Technical dossier/p. 25-26/Additional data July 2015.
[17] Technical dossier/p. 22, 46/Annex 1, 4 and 11-3/Additional data July 2015.

Table 1: Compositional data of the food enzyme preparation

Parameters	Unit	Batches			
		1	2	3	4[(a)]
α-Amylase activity	Units/g batch[(b)]	1,700	1,170	2,160	1,060
Protein	%	3.28	2.66	3.94	1.90
Ash	%	5.0	5.6	7.6	4.9
Water	%	58.2	56.1	55.0	92.8
Total stabilisers[(c)]	%	30.46	32.26	31.20	–
Total organic solids (TOS)[(d)]	%	6.34	6.04	6.20	2.30
Activity/mg TOS	Units/mg TOS	26.8	19.4	34.8	46.1

(a): Batch used for the toxicological studies.
(b): UNIT: α-Amylase activity (see Section 3.3.1).
(c): [redacted]
(d): TOS calculated as 100% – % water – % ash – % stabiliser.

3.3.3. Purity

The lead content in the three commercial batches and in the batch used for toxicological studies was below 5 mg/kg[18,19] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). In addition, the levels of mercury were below the limits of detection (LODs) of the employed method.[20,21] For arsenic and cadmium, the average concentration determined in the commercial batches were 0.043 and 0.012 mg/kg, respectively.[21,22] The Panel considered these concentrations as not of concern.

The food enzyme preparation complies with the microbiological criteria (for total coliforms, *Escherichia coli* and *Salmonella)* as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[23] No antimicrobial activity was detected in any of the tested 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 of the production strain

The absence of viable cells of the production strain in the food enzyme was demonstrated in [redacted] No colonies were produced.[24]

3.4. Toxicological data

A battery of toxicological tests, including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian chromosomal aberration test and a repeated dose 90-day oral toxicity study in rats, has been provided. The batch 4 (Table 1) used in these studies has a similar chemical purity to the batches intended for commercialisation, and thus is considered suitable as a test item.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was performed according to the Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 and 472 (OECD, 1995) and following Good Laboratory Practice (GLP).[25] Four strains of *Salmonella* Typhimurium (TA98, TA100,

[18] LOD: Pb = 0.005 mg/kg.
[19] Technical dossier/p. 23, 46/Annex 2.
[20] LOD: Hg = 0.001 mg/kg.
[21] Technical dossier/Annex 1 and 4.
[22] LODs: As = 0.002 mg/kg; Cd = 0.001 mg/kg.
[23] Technical dossier/p. 23/Annex 2 and 4.
[24] Technical dossier/Additional data February 2022/Annex 6.
[25] Technical dossier/Annex 9.

TA1535 and TA1537) and *Escherichia coli* WP2uvrA (pKM101) were used in the presence or absence of metabolic activation (S9-mix), applying the preincubation method. A preliminary experiment was performed employing a concentration range of 35–2,300 μg TOS/plate. Two separate experiments were then carried out in triplicate using five concentrations of the food enzyme (140, 290, 580, 1,150, 2,300 μg TOS/plate).[26] No cytotoxicity was observed at any concentration level of the test substance. Upon treatment with the food enzyme there was no significant increase in revertant colony numbers above the control values in any strain with or without S9-mix.

The Panel concluded that the food enzyme did not induce gene mutations under the test conditions employed in this study.

3.4.1.2. *In vitro* mammalian chromosomal aberration test

The *in vitro* mammalian chromosomal aberration test was carried out according to OECD Test Guideline 473 (OECD, 1983) and following GLP.[27] A dose-range finding experiment and a main experiment were performed in duplicate cultures of Chinese hamster lung cells.

The dose finding experiment was performed at a range of concentrations of food enzyme up to 6,900 μg TOS/mL. In a short-term treatment (6 h followed by 18 h recovery period) with and without metabolic activation (S9-mix) and a continuous treatment in the absence of S9-mix for 1.5 and 3 cell cycles. No inhibition of cell growth by 50% or more was observed in the short-term treatment, whereas 50% inhibition was observed in the continuous treatment at 3,400 and 1,000 μg TOS/mL at 1.5 and 3 cell cycles, respectively. Based on these results, in the main experiment the cells were exposed to the food enzyme at 860, 1,730, 3,450 and 6,900 μg TOS/mL, in the short-term treatment with and without metabolic activation (S9-mix) and at 220, 430, 860 and 1,730 μg TOS/mL in the continuous treatment for 1.5 cell cycles and 70, 140, 290 and 580 μg TOS/mL for 3 cell cycles in the absence of S9-mix.

The frequency of structural and numerical chromosomal aberrations, evaluated in 200 metaphases per concentration in treated cultures, was comparable to the values detected in negative controls.

The Panel concluded that food enzyme did not induce chromosome aberrations under the test conditions employed for this study.

3.4.2. Repeated dose 90-day oral toxicity study in rodents

The repeated dose 90-day oral toxicity study was performed in accordance with OECD Test Guideline 408 (a OECD, 1981) and following GLP.[28] Groups of 20 male and 20 female Sprague–Dawley rats received the food enzyme in doses of 57.5, 115 and 230 mg TOS/kg body weight (bw) per day by gavage. Controls received the vehicle (water). Furthermore, a recovery control and high-dose group were included in the study, each comprising 10 males and 10 females, and terminated 4 weeks after the end of treatment.

Five premature deaths were recorded in the main study: two males from the control group in weeks 5 and 7, respectively, one low-dose male in week 11, one mid-dose female in week 13 and one high-dose male in week 3. In four cases, death could be ascribed to gavage error. The cause of one spontaneous death in the control group was not identified. Furthermore, one recovery control female was killed during the blood sampling in week 13. The Panel considered the deaths as not related to the toxicity of test substance.

The body weight was statistically significantly decreased on administration days 7 (−3%), 21 (−5%), 28 (−5%), 35 (−5%), 42 (−6%), 49 (−6%), 56 (−6%), 63 (−5%), 70 (−6%) and 77 (−6%) in low-dose females and on administration days 21 (−5%), 28 (−5%), 35 (−5%), 42 (−6%), 49 (−6%), 56 (−8%), 63 (−6%), 70 (−7%) and 77 (−7%) in mid-dose females. The Panel considered the changes as not toxicologically relevant as they were only observed in one sex, there was no dose–response relationship, the magnitude of the changes was low and the changes were without a statistically significant effect on the final body weight.

The feed consumption was statistically significantly decreased on administration day 42 (−8%, −7% and − 5%) and 70 (−7%, −6% and − 3%) in low-, mid- and high-dose females, on day 49 (−8%) in mid-dose females, and on day 56 in low-dose females (−5%) and in mid-dose females (−7%). The Panel considered the changes as not toxicologically relevant as they were only observed in one sex, there was no dose–response relationship, the magnitude of the changes was low, there

[26] Technical dossier/Additional data July 2015.
[27] Technical dossier/Annex 10.
[28] Technical dossier/Annex 11.

were no statistically significant changes in the final feed consumption and there were no statistically significant changes in the final body weight.

The haematological investigation revealed a statistically significant increase in red blood cell (RBC) count in mid-dose males (+3%), in monocyte differential count in high-dose males (+26%) and in basophile differential count in mid- and high-dose males (+50% in both groups). Furthermore, a statistically significant increase in platelets (+25%) and prothrombin time (PT) (+6%) was recorded in recovery high-dose females. The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (all parameters), there was no dose–response relationship (RBC, basophils), the magnitude of the changes was low (RBC, monocytes, basophils, PT), the changes were not present at the end of the treatment period (platelets, PT) and there were no changes in other relevant parameters (platelets, PT).

The clinical chemistry investigation revealed a statistically significantly increase in the urea concentration in high-dose males (+9%) and a decrease in the concentration of triglycerides in low- and mid-dose females (−30% and −28%, respectively). In the recovery high-dose males, a statistically significantly increased activity of sorbitol dehydrogenase (SDH) was observed (+11%). The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (all parameters), there was no dose–response relationship (triglycerides), there were no histopathological changes in the kidneys (urea) and the changes were not seen at the end of the treatment period (SDH).

The urinalysis revealed a statistically significant increase in urine specific gravity (USG) in low dose males (+0.3%) and in low- and mid-dose females (+0.9% and + 1.3%), in urinary volume (UVol) in high-dose males (+300%) and high-dose females (+168%), in pH in low-dose males (+5%) and in low- and mid-dose females (+11%, +8%), and a decrease in pH in high-dose males (−8%), at 4 h sampling time. At 20 h sampling time, mid- and high-dose males excreted more sodium (+37%, +42%, respectively) and chloride (+34%, +31%, respectively). After the recovery period, the urine pH was statistically significantly increased (+12%) in high-dose females at 4 h sampling time and the urinary sodium concentration was statistically significantly lower (−17%) in the high-dose females at 20 h sampling time. The Panel considered the changes as not toxicologically relevant as there was no dose–response relationship (USG, pH), they were only observed in one sex (sodium and chloride at the end of the treatment, sodium at the end of recovery), the magnitude of the changes was low (USG), they were only recorded at one time point of the sampling (USG, UVol, pH), there was no consistency between the changes in males and females (sodium and chloride at the end of the treatment period, pH and sodium at the end of the recovery period) and there were no histopathological changes in the kidneys.

Statistically significant changes in organ weights included a decrease in absolute (−12%) and relative (−6%) weights of testes in mid-dose males, a decrease in parotid gland weight (−29% absolute and − 28% relative) in low-dose females and in mid-dose females (−20% absolute), an increase in thyroid absolute and relative weights (+21% for both) in high-dose females, a decrease in absolute and relative weights of uterus in low-dose females (−23% for both) and in high-dose females (−23% for both) and an increase in the relative weight of the liver in high-dose females (+5%). After the recovery period, increased absolute and relative weights of the liver (+11% and +13%, respectively) and of the prostate (+42% for both) were recorded in high-dose males. The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (parotid, thyroid, liver), there was no dose–response relationship (testes, parotid, uterus), the magnitude of the changes was low (testes, liver), the changes were not seen at the end of the treatment period (liver and prostate) and there were no histopathological changes in the organs (all organs).

No other statistically significant or biologically relevant differences to controls were reported.

The Panel identified the no observed adverse effect level (NOAEL) of 230 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity

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

The potential allergenicity of the α-amylase produced with *C. funkei* strain AE-AMT 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, nine matches were found.[29] The matching allergens were eight α-amylases and one glucoamylase produced by insects, mites or fungi, all known as respiratory allergens.

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

Several studies have shown that adults with asthma caused by respiratory allergens (as described for α-amylase from *A. oryzae*) may be able to ingest the corresponding allergen without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Poulsen, 2004; Armentia et al., 2009). Taking into account the wide use of α-amylase as a food enzyme only a low number of case reports of allergic reactions upon oral exposure to α-amylase in individuals respiratorily sensitised to α-amylase have been described in literature (Quirce et al., 1992; Losada et al., 1992; Baur and Czuppon, 1995; Kanny and Moneret-Vautrin, 1995; Moreno-Ancillo et al., 2004).

[redacted] a product that may cause allergies or intolerances (listed in the Regulation (EU) No 1169/2011[30]) is used as raw material. In addition, [redacted] and [redacted], known sources of allergens, are also present 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 proteins are not expected to be present in the food enzyme.[31]

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 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 to produce maltodextrin at the recommended use level of 22–109 mg TOS/kg starch.[32]

In starch processing, the food enzyme is added to starch during the liquefaction and saccharification steps.[33] The α-amylase, subject of this assessment, hydrolyses the α-1,4 bond in amylose to yield predominantly maltotriose. The food enzyme–TOS is considered to be removed from the maltotriose products by purification treatment with activated charcoal or similar, and with ion-exchange resins (EFSA CEP Panel, 2021b).

3.5.2. Dietary exposure estimation

In accordance with the guidance document (EFSA CEP Panel, 2021a), a dietary exposure was not calculated.

3.6. Margin of exposure

Since the estimation of a dietary exposure was considered unnecessary by the Panel, the margin of exposure was not calculated.

4. Conclusions

Based on the data provided and the removal of TOS during the intended food production process, the Panel concludes that the food enzyme α-amylase from *Cellulosimicrobium funkei* strain AE-AMT does not give rise to safety concerns under the intended conditions of use.

[29] Technical dossier/Additional data February 2022/Annex 4.

[30] 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.

[31] Technical dossier/Annex 7.

[32] Technical dossier/p. 41/Additional data February 2022.

[33] Technical dossier/p. 40.

5. Documentation as provided to EFSA

Application for authorisation of alpha-amylase from *Microbacterium imperiale* AE-AMT in accordance with the Regulation (EC) No 1331/2008. July 2014. Submitted by Amano Enzymes Inc.

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Abbreviations

bw	body weight
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
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practice
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kilodalton
LOD	limit of detection
NBRC	National Institute of Technology and Evaluation Biological Resource Center, Japan
OECD	Organisation for Economic Cooperation and Development
PT	prothrombin time
RBC	red blood cell
SDH	sorbitol dehydrogenase
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	total organic solids
WGS	whole genome sequence
WHO	World Health Organization