SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 12 December 2019

doi: 10.2903/j.efsa.2020.5976

Safety evaluation of the food enzyme α-amylase from *Bacillus amyloliquefaciens* strain BANSC

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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 *B. amyloliquefaciens* strain BANSC by Advanced Enzyme Technologies Ltd. The α-amylase is intended to be used in brewing and baking processes and in starch processing for glucose syrups production and other starch hydrolysates. Since residual amounts of the food enzyme are removed during the starch processing for glucose syrups production, it is excluded from the dietary exposure estimation. Based on the maximum recommended use levels for brewing and baking processes, and individual data from the EFSA Comprehensive European Food Database, dietary exposure to the food enzyme–Total Organic Solids (TOS) was estimated to be up to 0.468 mg TOS/kg body weight (bw) per day. The parental strain meets the required qualifications to be considered as a Qualified Presumption of Safety (QPS) organism and is therefore presumed to be safe. The conclusions on safety of the food enzyme are made following the QPS approach in relation to the production strain, with additional consideration of the conditions of manufacture. Consequently, the Panel considers no toxicological studies other than assessment of allergenicity 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 upon dietary exposure to this food enzyme cannot be excluded, but the likelihood is considered low. Based on the QPS status of the production strain and 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, 4-α-D-glucan glucanohydrolase, EC 3.2.1.1, 1,4-α-D-glucan glucanohydrolase, *Bacillus amyloliquefaciens*, genetically modified microorganism

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Question number: EFSA-Q-2014-00730

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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.

Suggested citation: EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Silano V, Barat Baviera JM, Bolognesi C, 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, Glandorf B, Herman L, Andryszkiewicz M, Arcella D, Gomes A, Liu Y and Chesson A, 2020. Scientific Opinion on the safety evaluation of the food enzyme α-amylase from *Bacillus amyloliquefaciens* strain BANSC. EFSA Journal 2020;18(1):5976, 14 pp. https://doi.org/10.2903/j.efsa.2020.5976

ISSN: 1831-4732

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

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1. Introduction

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

> 'Food enzyme' means a product obtained from plants, animals or micro-organisms or products thereof including a product obtained by a fermentation process using 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 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, and
iii) 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.

Four applications have been introduced by the companies 'Advanced Enzyme Technologies Ltd', 'DuPont Nutrition Biosciences ApS', 'Amano Enzyme Inc.' and 'Puratos NVsa' for the authorisation of the food enzymes Amylase from *Bacillus amyloliquefaciens* (strain BANSC), Beta-amylase from Barley (*Hordeum vulgare*), Triacylglycerol lipase from *Rhizopus niveus* (strain AE-N) and Xylanase from a genetically modified strain *Bacillus subtilis* TD160(229).

Following the requirements of Article 12.1 of Commission Regulation (EU) No 234/2011[3] implementing Regulation (EC) No 1331/2008, the Commission has verified that the four 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, p. 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, p. 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.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out a safety assessments of the food enzymes Amylase from *Bacillus amyloliquefaciens* (strain BANSC), Beta-amylase from Barley (*Hordeum vulgare*), Triacylglycerol lipase from *Rhizopus niveus* (strain AE-N) and Xylanase from a genetically modified strain *Bacillus subtilis* TD160(229) 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 of the safety assessment of the food enzyme α-amylase from *B. amyloliquefaciens* strain BANSC.

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 the non-genetically modified *B. amyloliquefaciens* strain BANSC.

Additional information was requested from the applicant during the risk assessment process on 15/05/2019 and was consequently provided (see 'Documentation provided to EFSA').

Following the request for additional data sent by EFSA on 15 May 2019, EFSA requested a clarification teleconference, which was held on 9 October 2019 and additional data were 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 guidances of EFSA Scientific Committee.

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 '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: Endo-amylase, 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-D-glucosidic linkages in polysaccharides (amylose and amylopectin), resulting in the generation of oligosaccharides. It is intended to be used in brewing and baking processes and in starch processing for glucose syrups production and other starch hydrolysates.

3.1. Source of the food enzyme

The α-amylase is produced with the non-genetically modified *B. amyloliquefaciens* strain BANSC, which is deposited at [redacted] with the deposit number [redacted][4]

The production strain was identified as *B. amyloliquefaciens* by 16S rRNA gene sequence analysis.

B. amyloliquefaciens 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). Cytotoxicity test was performed using Vero cells on the ten-fold concentrated culture supernatant of the production strain according to the

[4] Technical dossier/1st submission/Annex I1.

CEP Statement (EFSA CEP Panel, 2019). No cytotoxic effects were detected[5] and susceptibility to the battery antibiotics recommended by EFSA guidance (EFSA CEP Panel, 2019) was tested,[5] Minimum Inhibitory Concentration (MIC) values were always below cut-off values provided by EFSA.

The Panel concluded that the production strain met the criteria for QPS approach for the safety assessment, and thus can be presumed to be of no concern.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[6], with food safety procedures based on Hazard Analysis and Critical Control Points (HACCP), and in accordance with current Good Manufacturing Practice (GMP).

The food enzyme is grown as a pure culture using a typical industrial medium in contained, batch fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration leaving a supernatant containing the food enzyme. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in which enzyme protein is retained while most of the low molecular weight material passes the filtration membrane and is discarded. The applicant provided information on the identity of the substances used to control the fermentation and in the subsequent downstream processing of the food enzyme.[7]

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 514 amino acids.[8] The apparent molecular mass based on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) pattern is about 56 kDa[9] consistent with the expected mass of the enzyme. No other enzyme activities were reported.[10]

The in-house determination of α-amylase activity is based on the hydrolysis of starch, determined by comparing the iodine colour of the hydrolysate with that of the reference standard colour at 660 nm (reaction conditions: 30°C; 15 min, pH 3–9). One bacterial amylase unit (BAU) is defined as the amount of enzyme that will dextrinize starch at the rate of 1 mg/min under the standard assay conditions.[11]

The food enzyme has a temperature optimum around 60°C (pH 6.6) and a pH optimum between 5.0 and 6.5 (temperature 30°C). Thermostability was tested after an incubation of the food enzyme for 2 hours at different temperatures. Under the conditions (pH 6.6) of the applied temperature stability assay, the α-amylase activity decreased considerably above 70°C and showed no residual activity at 80°C.[12]

3.3.2. Chemical parameters

Data on chemical parameters of the food enzyme were provided for three commercial batches (Table 1).[13] The average total organic solids (TOS) content of the three commercial batches was 64%. The average enzyme activity/TOS ratio of the three batches used for commercialisation is 413.5 BAU/mg TOS.

[5] Technical dossier/1st submission/Annex I2.
[6] 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.
[7] Technical dossier/1st submission/p. 25-30; Technical dossier/1st submission/Annex G Conf.
[8] Technical dossier/1st submission/p. 6; Additional information August 2019/Annex 1.
[9] Technical dossier/1st submission/p. 5-6 and Annex B.
[10] Technical dossier/1st submission/p. 10.
[11] Technical dossier/1st submission/Annex C.
[12] Technical dossier/1st submission/p. 11-13 and Annex C.
[13] Technical dossier/1st submission/p. 5, 7-9 and Annex A2; Additional information July 2019.

Table 1: Compositional data provided for the food enzyme preparation

Parameter	Unit	Batch		
		1	2	3
α-Amylase activity	BAU/g batch[(a)]	267,110	255,426	270,125
Protein	%	43.12	40.71	44.56
Ash	%	7.98	8.24	7.35
Water	%	6.75	7.25	5.45
Total Organic Solids (TOS)[(b)]	%	65.27	59.51	67.2
α-Amylase BAU/mg TOS	BAU/mg TOS	409.24	429.22	401.97
Diluent	%	20	25	20

(a): BAU/g batch: Bacterial Amylase Unit (see Section 3.3.1).
(b): TOS calculated as 100% - % water - % ash - % diluent.

3.3.3. Purity

The lead content in three food commercial batches was below 0.25 mg/kg[14,15] 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). In addition, the levels of arsenic, cadmium and mercury were below the limits of detection (LODs) of the employed methodologies.[16,17]

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 present at not more than 30 colony forming units (CFU) per gram.[18] No antimicrobial activity was detected in any of these batches (FAO/WHO, 2006).[19]

The presence of mycotoxins (aflatoxins: B1, B2, G1, G2 and M1; ochratoxin A; zearalenone; deoxynivalenol (DON); T2-toxin; HT2-toxin; ergocornine; ergocristine; ergocryptine; ergometrine; ergosine; ergotamine) was examined in three commercial batches and were below the LOD of the applied analytical methods.[20,21]

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 production strain meets the criteria for QPS and is presumed safe. Consequently, in accordance with the CEP Statement (EFSA CEP Panel, 2019), these data are not needed.

3.4. Toxicological data

No toxicological tests were provided by the applicant. The Panel considers no toxicological studies other than assessment of allergenicity necessary. This is based on the QPS status of the production strain (see Section 3.1) and the absence of any hazards arising from the manufacturing of the 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.

The potential allergenicity of the α-amylase produced with the non-genetically modified *B. amyloliquefaciens* strain BANSC was assessed by comparing its amino acid sequence with those of

[14] LOD: Pb = 0.25 mg/kg.
[15] Technical dossier/1st submission/p. 7; Technical dossier/1st submission/Annex D and A2.
[16] LOD: As = 0.1 mg/kg, Cd = 0.1 mg/kg, Hg = 0.025 mg/kg.
[17] Technical dossier/1st submission/Letter_TUV_LOD_Heavy Metals.
[18] Technical dossier/1st submission/p. 10; Technical dossier/1st submission/Annex A2; Letter_TNO_LOD_Mycotoxin.
[19] Technical dossier/1st submission/p. 7; Technical dossier/1st submission/Annex A2.
[20] LOD: aflatoxin B1 = 1 μg/kg; aflatoxin B2 = 1 μg/kg; aflatoxin G1 = 1 μg/kg; aflatoxin G2 = 1 μg/kg; aflatoxin M1 = 1 μg/kg; ochratoxin A = 1 μg/kg; zearalenone = 5 μg/kg; DON = 25 μg/kg; T2-toxin = 10 μg/kg; HT2-toxin = 50 μg/kg; ergocornine = 100 μg/kg; ergocristine = 100 μg/kg; ergocryptine = 100 μg/kg; ergometrine = 100 μg/kg; ergosine = 100 μg/kg; ergotamine = 100 μg/kg.
[21] Technical dossier/1st submission/p. 10 Technical dossier/1st submission/Annex E.

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, 2017). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, one match was found.[22] The matching allergen was Asp o 21, an α-amylase produced by *Aspergillus oryzae*.

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

α-Amylase from *A. oryzae* is not identified as a food allergen by both the AllergenOnline[23] and the WHO/IUIS allergen nomenclature subcommittee database.[24] α-Amylase from *A. oryzae* (Brisman and Belin, 1991; Sander et al., 1998; Brisman, 2002; Quirce et al., 2002) is described as occupational respiratory allergens associated with baker's asthma. However, several studies have shown that adults with occupational asthma to a food 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 have been described in the literature focused on allergic reactions upon oral exposure to α-amylase in individuals respiratory 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).

According to the information provided, substances or products that may cause allergies or intolerances (Regulation (EU) No 1169/2011[25]) are used as raw materials ([redacted]) 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.

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 three food processes. Intended uses and the recommended use levels are summarised in Table 2.[26]

Table 2: Intended uses and recommended use levels of the food enzyme as provided by the applicant

Food manufacturing process[(a)]	Raw material	Recommended dosage of the food enzyme
Brewing processes	Cereals	Up to 102 mg TOS/kg cereals
Baking processes	Flour	Up to 0.29 mg TOS/kg flour
Starch processing for glucose syrups production and other starch hydrolysates	Starch	Up to 173 mg TOS/kg starch

TOS: total organic solids.

(a): The description provided by the applicant has been harmonized by EFSA according to the 'EC working document describing the food processes in which food enzymes are intended to be used' – not yet published at the time of adoption of this opinion.

[22] Additional information July 2019/Annexure 2.

[23] http://www.allergenonline.org

[24] http://allergen.org

[25] 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.

[26] Technical dossier/1st submission/p. 31–37; Additional information August 2019; Additional information November 2019.

In brewing processes, the α-amylase is added during the mashing step or to the adjunct before the addition of the adjunct to the mash tun. The α-amylase is used to convert liquefied starch into a maltose-rich solution, improving the amounts of fermentable sugars and thus increasing brewing yield.

In baking processes, the food enzyme is added to flour during the preparation of dough. The α-amylase hydrolyses starch from granules that have been damaged during milling and release fermentable sugars and dextrins. This reaction shortens the processing time and decreases dough viscosity. The latter facilitates the handling of the dough, resulting in more uniform products with better properties (increased firmness, reduced oil absorption and less stockiness).

In starch processing for glucose syrups production and other starch hydrolysates, the food enzyme is used for raw starch hydrolysis to produce glucose, maltose syrups and maltodextrins.

Experimental data have been provided on the removal (> 99%) of protein in the course of starch processing for glucose syrups production (Documentation provided to EFSA No 3). The Panel considered the evidence as sufficient to conclude that residual amounts of TOS are removed by the purification steps applied to the production of glucose syrups (i.e. filtration, ion exchange chromatography, treatment with active carbon) to a similar degree.

In the view of the Panel, the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) data can be used to include maltodextrins because of essentially similar production method (Annex B in EFSA CEF Panel, 2016).

The food enzyme remains in the beer and dough. Based on data provided on thermostability (see Section 3.3.1), it is expected that the enzyme is inactivated during brewing and baking processes.

3.5.2. Dietary exposure estimation

As residual amounts of TOS are removed by the purification steps applied during starch processing for glucose syrups production and other starch hydrolysates (see Section 3.5.1), foods/ingredients derived through this process, i.e. glucose and maltose syrups and maltodextrins, were excluded from the estimation.

For the baking and brewing processes, chronic exposure was calculated using the methodology described in the 'CEF Panel statement on the exposure assessment of food enzymes' (EFSA CEF Panel, 2016). The assessment involved selection of relevant food categories from the EFSA Comprehensive European Food Consumption Database and application of process and technical conversion factors (Annex B in EFSA CEF Panel, 2016).

Chronic exposure was calculated by combining the maximum recommended use level provided by the applicant (see Table 2) with the relevant FoodEx categories (Annex B in EFSA CEF Panel, 2016), based on individual consumption data. Exposure from individual FoodEx categories was subsequently summed up, averaged over the total survey period and normalised for bodyweight. This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only one day per subject were excluded and high-level exposure/intake was calculated for only those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011).

Table 3 provides an overview of the derived exposure estimates across all surveys. Detailed average and 95th percentile exposure to the food enzyme–TOS per age class, country and survey, as well as contribution from each FoodEx category to the total dietary exposure are reported in Appendix A – Tables 1 and 2. For the present assessment, food consumption data were available from 35 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B).

Table 3: Summary of estimated dietary exposure to food enzyme–TOS in six population groups

Population group	Estimated exposure (mg TOS/kg body weight per day)					
	Infants	**Toddlers**	**Children**	**Adolescents**	**Adults**	**The elderly**
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0.000–0.001 (10)	0.001–0.002 (14)	0.001–0.003 (19)	0.000–0.020 (18)	0.008–0.104 (19)	0.003–0.052 (18)
Min–max 95th percentile (number of surveys)	0.000–0.003 (8)	0.002–0.003 (12)	0.001–0.003 (19)	0.001–0.124 (17)	0.058–0.468 (19)	0.013–0.214 (18)

TOS: total organic solids.

Based on the maximum use levels recommended for brewing and baking processes and individual data from the EFSA Comprehensive European Food Database, dietary exposure to the food enzyme–TOS was estimated to be up to 0.468 mg TOS/kg bw per day.

3.5.3. Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the following sources of uncertainties have been considered and are summarised in Table 4.

Table 4: Qualitative evaluation of the influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction of impact
Model input data	
Consumption data: different methodologies/representativeness/underreporting/ misreporting/no portion size standard	+/–
Use of data from food consumption surveys of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Possible national differences in categorisation and classification of food	+/–
Model assumptions and factors	
FoodEx categories included in the exposure assessment were assumed to always contain the food enzyme–TOS	+
Exposure to food enzyme–TOS was always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
Use of recipe fractions in disaggregation FoodEx categories	+/–
Use of technical factors in the exposure model	+/–
Exclusion of other processes from the exposure estimate: – starch processing for glucose syrups production and other starch hydrolysates	–

TOS: total organic solids.
+: uncertainty with potential to cause overestimation of exposure.
–: uncertainty with potential to cause underestimation of exposure.

The conservative approach applied to the exposure estimate to food enzyme–TOS in brewing and baking processes, in particular assumptions made on the occurrence and use levels of this specific food enzyme, is likely to have led to a considerable overestimation of the exposure.

The exclusion of one food manufacturing process (starch processing for glucose syrups production and other starch hydrolysates – see Table 4) from the exposure assessment was based on > 99% of TOS removal during this process and is not expected to have an impact on the overall estimate derived.

Conclusions

Based on the QPS status of the production strain and the data provided, the Panel concluded that the food enzyme α-amylase produced with the non-genetically modified strain *B. amyloliquefaciens* strain BANSC does not give rise to safety concerns under the intended conditions of use.

Documentation provided to EFSA

1) Dossier 'Application for authorisation of Alpha Amylase from *Bacillus amyloliquefaciens* strain BANSC in accordance with the Regulation (EC) No 1331/2008'. September 2014. Submitted by Advanced Enzyme Technologies Ltd.
2) Summary report on technical data and dietary exposure related to alpha-amylase from *Bacillus amyloliquefaciens* (strain BANSC) by Advanced Enzyme Technologies Ltd. March 2015. Delivered by Hylobates Consulting and BiCT.
3) Additional information. August 2019. Submitted by Advanced Enzyme Technologies Ltd.
4) Additional information. November 2019. Submitted by Advanced Enzyme Technologies Ltd.

5) Additional information on 'Grain processing/Fate of the food enzymes'. 26 April 2018 and 13 July 2018. Provided by the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) and Starch Europe. Unpublished document.

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Abbreviations

AMFEP	Association of Manufacturers and Formulators of Enzyme Products
BAU	Bacterial Amylase Unit
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 unit
DON	deoxynivalenol
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen
EC	Enzyme Commission
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GMO	genetically modified organism
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points
IUBMB	International Union of Biochemistry and Molecular Biology
IUIS	International Union of Immunological Societies
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
MIC	Minimum Inhibitory Concentration
QPS	Qualified Presumption of Safety
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	Total Organic Solids
WHO	World Health Organization

Appendix A – Dietary exposure estimates to the food enzyme–TOS in details

Information provided in this appendix is shown in an excel file (downloadable https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2020.5976).

The file contains two sheets, corresponding to two tables.

Table 1: Average and 95th percentile exposure to the food enzyme–TOS per age class, country and survey.

Table 2: Contribution of food categories to the dietary exposure to the food enzyme–TOS per age class, country and survey.

Appendix B – Population groups considered for the exposure assessment

Population	Age range	Countries with food consumption surveys covering more than one day
Infants	From 12 weeks on up to and including 11 months of age	Bulgaria, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, United Kingdom
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Spain, United Kingdom
Children[(a)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Portugal, Spain, Sweden, United Kingdom
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Spain, Sweden, United Kingdom
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Spain, Sweden, United Kingdom
The elderly[(a)]	From 65 years of age and older	Austria, Belgium, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Spain, Sweden, United Kingdom

(a): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011).