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SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 27 January 2021

doi: 10.2903/j.efsa.2021.6433

Safety evaluation of the food enzyme α-amylase from the genetically modified *Bacillus licheniformis* strain NZYM-KE

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Abstract

The food enzyme α-amylase (4-α-D-glucan glucanohydrolase; EC 3.2.1.1) is produced with the genetically modified *Bacillus licheniformis* strain NZYM-KE by Novozymes A/S. The genetic modifications do not give rise to safety concerns. The food enzyme is free from viable cells of the production organism and its DNA. The α-amylase is intended to be used in starch processing for the production of glucose syrups and other starch hydrolysates, and distilled alcohol production. Since residual amounts of the food enzyme are removed by the purification steps applied during the production of glucose syrups and distillation, no dietary exposure was calculated. 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 1,100 mg TOS/kg body weight (bw) per day. A search for similarity of the amino acid sequence of the food enzyme to known allergens was made and one match was found. The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but the likelihood is considered to be low. Based on the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

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

Requestor: European Commission

Question number: EFSA-Q-2012-00898

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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.

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, Lambré C, 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, Andryszkiewicz M, Liu Y, Ranieri S and Chesson A, 2021. Scientific Opinion on the safety evaluation of the food enzyme α-amylase from the genetically modified *Bacillus licheniformis* strain NZYM-KE. EFSA Journal 2021;19(3):6433, 14 pp. https://doi.org/10.2903/j.efsa.2021.6433

ISSN: 1831-4732

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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 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.

Two applications have been introduced by the company Novozymes A/S for the authorisation of the food enzymes Alpha-amylase from a genetically modified strain of *Bacillus licherniformis* (strain NZYM-KE) and Xylanase from a genetically modified strain of *Aspergillus oryzae* (strain NZYM-FB).

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 both 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

In accordance with the requirements of Article 12.2 of Regulation (EC) No 234/2011, the Commission requests the European Food Safety Authority to verify the suitability of the data for risk assessment of both applications within 30 working days following the receipt of the Commission's request.

[1] Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on Food Enzymes and Amending Council Directive 83/417/EEC, Council Regulation (EC) No. 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L 354, 31.12.2008, pp. 7–15.

[2] Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, pp. 1–6.

[3] Commission Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 64, 11.3.2011, pp. 15–24.

If the data of the applications are considered suitable for risk assessment, the European Food Safety Authority should prepare opinions about the safety of the two food enzymes within 18 months.

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 a genetically modified *Bacillus licherniformis* (strain NZYM-KE).

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 a genetically modified *Bacillus licherniformis* (strain NZYM-KE).

Additional information was requested from the applicant during the assessment process on 21 June 2013, 13 June 2014 and 20 October 2020 and was consequently provided (see 'Documentation provided to EFSA').

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA Guidance on transparency in the scientific aspects of risk assessment (EFSA, 2009b) as well as in the Statement on characterisation of microorganisms used for the production of food enzymes (EFSA CEP Panel, 2019) and following the relevant existing guidance's of EFSA Scientific Committees.

The current Guidance on the submission of a dossier on food enzymes for safety evaluation (EFSA, 2009a) has been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance to the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016).

3. Assessment

IUBMB nomenclature	α-Amylase
Systematic name	4-α-D-glucan glucanohydrolase
Synonyms	Glycogenase, endoamylase, 1,4-α-D-glucan glucanohydrolase, 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 enzyme is intended to be used in starch processing for the production of glucose syrups and other starch hydrolysates, and distilled alcohol production.

3.1. Source of the food enzyme

The α-amylase is produced with the genetically modified bacterium *Bacillus licheniformis* strain NZYM-KE, which is deposited in the German Collection of Microorganisms and Cell Cultures (DSMZ, Germany) with deposit number [redacted].[4] The strain was identified as *B. licheniformis* by [redacted].[5]

The species *B. licheniformis* is included in the list of organisms for which the qualified presumption of safety (QPS) may be applied, provided that the absence of acquired antimicrobial resistance (AMR) genes and toxigenic activity are verified for the specific strain used.[6] The absence of cytotoxic activity was confirmed using VERO cells.[5] [redacted] did not identify known genes encoding AMR.[5]

[4] Technical dossier/GMM dossier-Annex 4/Annex A4.
[5] Technical dossier/Additional information December 2020.
[6] https://zenodo.org/record/3336268#.YCpdCuhKiUn

3.1.1. Characteristics of the parental and recipient microorganisms

The parental strain is *B. licheniformis* Ca63. The strain shows no cytotoxic activity in Chinese hamster ovary cells (Pedersen et al., 2002). The absence of cytotoxicity has also been shown by the same methodology in strain SJ1707, an intermediate strain (Pedersen et al., 2002).

.[8]

3.1.2. Characteristics of introduced sequences

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3.1.3. Description of the genetic modification process

[7] Technical dossier/GMM dossier-Annex 4/Annexes B22-B28.
[8] Technical dossier/GMM dossier-Annex 4/Annex D2.

.[10]

3.1.4. Safety aspects of the genetic modification

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

The production strain *B. licheniformis* NZYM-KE differs from the recipient strain only in its capacity to produce the α-amylase .[8]

The identity of the production strain is established as *B. licheniformis*, a species included in the QPS list. As it was shown to be free of AMR genes, not to be cytotoxic and as the genetic modifications applied did not raise concerns, the production stain is considered to meet the requirement of the QPS approach.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[11], with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[12]

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 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.[13] 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.[14]

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.

[9] Technical dossier/GMM dossier-Annex 4/Annex D1.

[10] Technical dossier/GMM dossier-Annex 4/Annexes C5-C8.

[11] 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.

[12] Technical dossier/page 74/Annex 5.

[13] Technical dossier/page 74–80.

[14] Technical dossier/Annex 6; Additional data December 2020/Annex_ Answers add. info_2012-00898_alpha-amylase_NZYM-KE.

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The α-amylase is a single polypeptide chain of 483 amino acids.[15] The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be 54.8 kDa.[16] The food enzyme was analysed by SDS–PAGE analysis. A consistent protein pattern was observed across all batches. The gels showed the target protein migrating between the marker proteins of 45 and 66 kDa in all batches, consistent with the calculated mass of the enzyme.[17] The food enzyme was tested for protease (acid, neutral and alkaline), β-glucanase, lipase, peroxidase, cellulase and amyloglucosidase activities and none were detected.[18]

The in-house determination of α-amylase activity is based on hydrolysis of the substrate 4,6-ethylidene(G7)-*p*-nitrophenyl(G1)-α-D-maltoheptaoside (ethylidene-G7pNP) combined with excess levels of α-glucosidase (reaction conditions: pH 7.0, temperature 37°C, reaction time 5 min). The analytical principle is based on the reaction between *p*-nitrophenyl maltosaccharide fragments (formed from the reaction between the substrate and the α-amylase) and the α-glucosidase, which liberates *p*-nitrophenol, which is determined spectrophotometrically at 405 nm. The activity is quantified relative to an internal enzyme standard and expressed in Kilo Novo alpha-amylase Units/g (KNU(B)/g).[19]

The food enzyme has a temperature optimum around 70°C (pH 5.5) and a pH optimum around pH 6–7 (30°C). Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures (pH 5.5). α-Amylase activity decreased above 60°C showing no residual activity above 80°C.[20] In a second experiment, no residual activity was observed after 90 seconds of incubation at 95°C (pH 5.5) using insoluble blue-dyed cross-linked starch as substrate.[21,22]

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).[23] The average total organic solids (TOS) of the three food enzyme batches for commercialisation was 9.5% and the average enzyme activity/TOS ratio was 14.7 KNU(B)/mg TOS.

Table 1: Compositional data of the food enzyme

Parameters	Unit	Batch			
		1	2	3	4[(a)]
α-amylase activity	KNU(B)/g batch[(b)]	1,320	1,490	1,370	1,670
Protein	%	7.5	7.8	7.6	9.0
Ash	%	1.8	2.2	2.0	2.7
Water	%	88.7	88.4	88.4	86.9
Total organic solids (TOS)[(c)]	%	9.5	9.4	9.6	10.4
Activity/mg TOS	KNU(B)/mg TOS	13.9	15.9	14.3	16.1

(a): Batch used for the toxicological studies.
(b): KNU(B): Kilo Novo alpha-amylase Units (see Section 3.3.1).
(c): TOS calculated as 100% – % water – % ash.

3.3.3. Purity

The lead content in the three commercial batches and in the batch used for toxicological studies was below 0.5 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). In

[15] Technical dossier/page 59/Annex 1.
[16] Technical dossier/page 59.
[17] Technical dossier/page 61.
[18] Technical dossier/page 68; Additional data August 2013.
[19] Technical dossier/Annex 3.
[20] Technical dossier/pages 67–68/Annex 9.
[21] Technical dossier/Additional data August 2013/Annex 3.02.
[22] Technical dossier/Additional information December 2020/Temp stability alpha-amylase NZYM-KE, Vers 2.
[23] Technical dossier/Additional information December 2020/CoA production batches NZYM-KE.

addition, the levels of cadmium, mercury and arsenic were below the limits of detection of the employed methodologies.[24,25]

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 should not exceed 30 colony forming units per gram. No antimicrobial activity was detected in any of the tested batches (FAO/WHO, 2006).[25]

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

3.3.4. Viable cells and DNA of the production strain

The absence of viable cells of the production strain in the food enzyme was demonstrated .[26]

The absence of recombinant DNA in the food enzyme was demonstrated .[27]

3.4. Toxicological data

As the production strain qualifies for the QPS approach of safety assessment and as no issue of concern arising from the production process of the food enzyme were identified (see Sections 3.1, 3.2 and 3.3), the Panel considers that no toxicological studies other than assessment of allergenicity are necessary. A battery of toxicological tests including a bacterial gene mutation assay (Ames test), an *in vitro* micronucleus assay and a repeated dose 90-day oral toxicity study in rats has been provided. The batch 4 (Table 1) used in these studies has similar protein pattern and chemical purity as the batches used for commercialisation, and thus is considered suitable as a test item. The tests are reported as supporting evidence of the safety of the food enzyme.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was performed according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997) and following Good Laboratory Practice (GLP).[28] Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *E. coli* WP2uvrA(pKM101) were used in the presence or absence of metabolic activation, applying the 'treat and plate' assay. Two separate experiments were carried out in triplicate using six different concentrations of the food enzyme (156, 313, 625, 1,250, 2,500 and 5,000 μg food enzyme/plate, corresponding to 16.2, 32.5, 65, 130, 260 and 520 μg TOS/plate). Cytotoxicity, evaluated as a reduction of the number of viable cells was observed in a single experiment in TA100 and TA1535 at the highest concentration tested in the absence of S9 mix. Growth stimulation, as demonstrated by increases in the viable cell count of the exposed cultures compared to the solvent control, was observed especially in the presence of S9-mix. Upon treatment with the food enzyme a significant increase in revertant colony numbers above the control values was observed in a single experiment in the strain TA1535 without S9-mix at the highest concentration tested (520 μg TOS/plate) and with S9-mix at 65 and 130 μg TOS/plate. However, the results were not dose dependent and not reproducible.

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

[24] LoDs: Pb = 0.5 mg/kg; As = 0.1 mg/kg; Cd = 0.05 mg/kg; Hg = 0.03 mg/kg.
[25] Additional information December 2020/CoA production batches NZYM-KE.
[26] Technical dossier/GMM dossier-Annex 4/Annex E1.
[27] Technical dossier/GMM dossier-Annex 4/Annex E2.
[28] Technical dossier/Annex 7.1.

3.4.1.2. *In vitro* micronucleus assay

The *in vitro* micronucleus test was carried out according to OECD Draft Guideline 487 (OECD, 2010) and following GLP.[29] An experiment was performed in duplicate cultures of human peripheral whole blood lymphocytes. Cells were exposed to the test substance for 3 hours in the presence or absence of the S9-mix and harvested 24 h after the beginning of treatment (3 + 21-h treatment). Additionally, a continuous 24-h treatment without S9 mix was included with harvesting 24 hours after removal of the test substance (24 + 24-h treatment). The food enzyme was tested at 3,000, 4,000 and 5,000 μg/mL (312, 416 and 520 μg TOS/mL) in the presence or in the absence of S9 mix in the short exposure assay and at 50, 200, 500 and 1,000 μg/mL of food enzyme (5.2, 20.8, 52 and 104 μg TOS/mL) in the 24-h exposure assay. The maximum concentration tested in the 24-h exposure assay was determined by evidence of cytotoxicity (50% reduction of the replication index) seen with 104 μg TOS/mL. The frequency of binucleated cells with micronuclei (MNBN) was comparable to the negative controls at all concentrations tested in the short-term treatment in the presence of S-9-mix. In the short treatment in the absence of S9-mix, a statistically significant increase of the frequency of MNBN with respect to the control was observed at 520 μg TOS/mL. In the 24-h treatment a statistically significant increase of MNBN was observed at three concentrations tested without a dose response effect. All the values were within the historical control range and were not considered biologically relevant.

The Panel concluded that the food enzyme α-amylase did not induce an increase in the frequency of MNBN in cultured human peripheral blood lymphocytes, under the test conditions employed in 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 (OECD, 1998) and following GLP.[30] Groups of 10 male and 10 female Sprague-Dawley (Crl:CD) rats received by gavage the food enzyme in 3 doses corresponding to 110, 362 and 1,100 mg TOS/kg body weight (bw) per day. Controls received the vehicle (water).

One high-dose female was killed in week 7 after suffering an injury that occurred by jumping off the scale during body weight measurement.

Among the functional observation battery tests, a statistically significant lower hindlimb strength was observed in mid- and high-dose females compared to the control group. However, these values were within the historical control range. Motor activity low beam break scores at 6 minutes were statistically significantly higher in high-dose males and at 30 and 36 min they were statistically significantly lower for all treated males. For females, high beam scores were statistically significantly higher at 30 min in mid- and high-dose groups with respect to the controls and low beam scores were statistically significantly higher in all treated females with respect to the controls. As no dose relationship could be identified, all these changes were considered as incidental.

Haematological investigation revealed a statistically significantly higher mean absolute count of basophiles in all treated males and in high-dose females. The high-dose females also had a statistically significant higher mean absolute count of white blood cells (WBC) and lymphocytes, and a statistically significantly lower mean cell haemoglobin concentration. Activated partial thromboplastin time (APTT) was statistically significantly higher in all treated females and in low and mid-dose males. Mid- and high-dose males also showed statistically significantly higher values of prothrombin time with respect to the controls. The Panel noted that the lymphocyte count of the concurrent control female group was in the lower bound of the 95 percentile of the historical control data presented by the authors of the study and that the low lymphocyte count was reflected in the low total WBC count in this group while the white blood cell and lymphocyte counts of the treated females were within the range of the historical control data. Subsequently, the Panel considered that the differences in WBC and lymphocyte counts in the high-dose females were of no toxicological relevance. Other differences relative to the control group were small (absolute basophil count, haemoglobin concentration, prothrombin time) or lacked dose–response relationship (APTT) and were considered not to be of toxicological relevance.

Clinical chemistry investigation revealed that high-dose males had a statistically significantly lower concentration of sodium and a statistically significantly higher concentration of potassium with respect to the control. All treated male groups also showed a statistically significant increase in the concentration of glucose and a statistically significant decrease in the concentration of alanine

[29] Technical dossier/Annex 7.2.
[30] Technical dossier/Annex 7.3.

aminotransferase (ALT). The changes in blood chemistry parameters were considered by the Panel as not toxicologically relevant because the differences were small (sodium and potassium) or without an apparent dose dependency (ALT) and were restricted to one sex. The increase in glucose concentration in all treated male groups was considered of not toxicological relevance because the control and the treated groups' values were within the 90 percentile range of the historical control data presented by the authors of the report.

There was a small but statistically significant decrease in the mean adjusted spleen weight in high-dose females. As this change was small, and no toxicologically relevant effects were seen in circulating white blood cells and thymus, the change in spleen weight was also not considered of toxicological relevance.

Microscopic examination revealed an increased incidence of submucosal inflammatory cell infiltration in the trachea of high-dose males (7/10 vs. 1/10). These lesions were also seen in both the control and high-dose females. The Panel considered these lesions could be related to irritation caused by improper gavage technique and aspiration of the test solution into the upper part of the respiratory tract.

No other statistically significant differences to controls were observed.

The Panel identified the no observed adverse effect level (NOAEL) of 1,100 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 the genetically modified *B. licheniformis* strain NZYM-KE 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, one match was found. The matching allergen was Asp o 21, an α-amylase produced by *Aspergillus oryzae* known as an occupational respiratory allergen.[31]

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

α-Amylase from *A. oryzae* (Brisman and Belin, 1991; Sander et al., 1998; Quirce et al., 2002; Brisman, 2002) is known as an occupational respiratory allergen associated with baker's asthma. However, several studies have 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 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[32]) 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.

Quantifying the risk for allergenicity is not possible in view of the individual susceptibility to food allergens. Allergenicity can be ruled out only if the proteins are fully removed as in the case of distilled alcohol production. In the starch processing for the production of glucose syrups, experimental data showed a significant removal (> 99%) of protein. However, traces of protein could be present in glucose syrup.

[31] Technical Dossier/Annex 8.

[32] 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.

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 can be excluded for distilled alcohol production. The risk cannot be excluded for starch processing for glucose syrups production, 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 food enzyme is intended to be used in two food manufacturing processes at the recommended use levels summarised in Table 2.

Table 2: Intended uses and recommended use levels of the food enzyme as provided by the applicant[33]

Food manufacturing process[(a)]	Raw material	Recommended dosage of the food enzyme
Starch processing for the production of glucose syrups and other starch hydrolysates	Starch	Up to 32.7 mg TOS/kg starch
Distilled alcohol production	Starch	Up to 32.7 mg TOS/kg starch

(a): The description provided by the applicant has been harmonised 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 adoption of this opinion.

In starch processing, the food enzyme is typically added during the saccharification step where it degrades gelatinised starch into dextrins.[34]

In distilled alcohol production, the food enzyme is added during the slurry mixing step, in the liquefaction step and, if needed, in the pre-saccharification step. It is intended to convert liquefied starch into a maltose-rich solution, to increase the amounts of fermentable sugars, which results in higher alcohol yields.[34]

3.5.2. Dietary exposure estimation

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

4. Conclusions

Based on the data provided and the removal of TOS during distilled alcohol production and starch processing, the Panel concluded that the food enzyme α-amylase produced with the genetically modified *B. licheniformis* strain NZYM-KE does not give rise to safety concerns under the intended conditions of use.

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

5. Documentation as provided to EFSA (if appropriate)

1) Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-KE) October 2012. Submitted by Novozymes A/S.
2) Additional information. August 2013. Submitted by Novozymes A/S.
3) Additional information. January 2015. Submitted by Novozymes A/S.
4) Additional information. December 2020. Submitted by Novozymes A/S.

[33] Additional information December 2020.
[34] Technical dossier/pp. 103–107.

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Abbreviations

ALT	alanine aminotransferase
AMR	antimicrobial resistance
APTT	activated partial thromboplastin time
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
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practice
IUBMB	International Union of Biochemistry and Molecular Biology
LoD	limit of detection
MNBN	binucleated cells with micronuclei
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
QPS	qualified presumption of safety
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	total organic solids
WBC	white blood cells
WGS	whole genome sequence
WHO	World Health Organization