Safety evaluation of the food enzyme alpha-amylase from a genetically modified Bacillus licheniformis (strain NZYM-AN)

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Safety evaluation of the food enzyme alpha-amylase from a genetically modified *Bacillus licheniformis* (strain NZYM-AN)

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Abstract

The food enzyme is an α-amylase (4-α-D-glucan glucanohydrolase; EC 3.2.1.1) produced with a genetically modified *Bacillus licheniformis* strain NZYM-AN by Novozymes A/S. The genetic modifications do not give rise to safety concerns. The food enzyme does not contain the production organism or recombinant DNA; therefore, there is no safety concern for the environment. The α-amylase is intended to be used in starch processing for the production of glucose syrups and distilled alcohol production. Residual amounts of total organic solids (TOS) are removed by distillation and by the purification steps applied during the production of glucose syrups (by > 99%). Consequently, dietary exposure was not calculated. Genotoxicity tests with the food enzyme did not raise a safety concern. The amino acid sequence of the food enzyme did not match to those of known allergens. The Panel considered that under the intended condition 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 microbial source, the genetic modifications, the manufacturing process, the compositional and biochemical data, the removal of TOS during the intended food production processes and the findings in the genotoxicity studies, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

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

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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 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:

1) it does not pose a safety concern to the health of the consumer at the level of use proposed,
2) there is a reasonable technological need, and
3) its use does not mislead the consumer.

All food enzymes currently on the EU market and intended to remain on that market as well as all new food enzymes shall be subjected to a safety evaluation by the European Food Safety Authority (EFSA) and approval via an EU Community list.

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

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

1.1.1. Background as provided by the European Commission

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.

Five applications have been introduced by the companies 'Roquette', 'Novozymes A/S', 'DSM Food Specialities B.V.' and 'Advanced Enzyme Technologies Ltd.' For the authorisation of the food enzymes Beta-amylase from wheat (*Triticum spp*), Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-AN), Chymosin from a genetically modified strain of *Kluyveromyces lactis* (strain CIN), Polygalacturonase from a genetically modified strain of *Aspergillus niger* (strain FLYSC) and Pectinestrerase from a genetically modified strain of *Aspergillus niger* (strain FLZSC).

Following the requirements of Article 12.1 of Commission Regulation (EU) No 234/2011[3] implementing Regulation (EC) No 1331/2008[2], the Commission has verified that the five 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 a safety assessments of the food enzymes Beta-amylase from wheat (*Triticum spp*), Alpha-amylase from a

[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.

genetically modified strain of *Bacillus licheniformis* (strain NZYM-AN), Chymosin from a genetically modified strain of *Kluyveromyces lactis* (strain CIN), Polygalacturonase from a genetically modified strain of *Aspergillus niger* (strain FLYSC) and Pectinestrerase from a genetically modified strain of *Aspergillus niger* (strain FLZSC) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. Interpretation of Terms of Reference

The present scientific opinion addresses the European Commission request to carry out of the safety assessment of the food enzyme alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-AN).

1.3. Information on existing authorisations and evaluations

The applicant reports that the authorities of France and Denmark have evaluated and authorised the use of α-amylase produced by genetically modified strains of *Bacillus licheniformis* in a number of food and beverage manufacturing processes (i.e. beverage alcohol production, starch processing, brewing processes) and specified the conditions of use.

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 licheniformis* (strain NZYM-AN). The food enzyme is intended to be used in two food-manufacturing processes: starch processing for the production of glucose syrups as well as in distilled alcohol production.

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA 'Guidance on transparency in the scientific aspects of risk assessment' (EFSA, 2009) and following the relevant existing guidances from the EFSA Scientific Committee.

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

3. Assessment

3.1. Technical data

3.1.1. Identity of the food enzyme

IUBMB nomenclature:	α-amylase
Systematic name:	4-α-D-glucan glucanohydrolase
Synonyms:	glycogenase, 1,4-α-D-glucan glucanohydrolase
IUBMB No:	EC 3.2.1.1
CAS No:	9000-90-2
EINECS No:	232-565-6

3.1.2. Chemical parameters

The molecular mass of 55.2 kDa reported in the dossier for the α-amylase produced by *Bacillus licheniformis* (strain NZYM-AN) was calculated from the amino acid sequence and was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The SDS-PAGE gel consistently showed a single major protein band in all batches.

Other enzymes (glucoamylase, lipase and protease) were analysed in three commercial food enzyme batches and were below the limits of detection of the applied assays.

Data on chemical parameters of the food enzyme have been provided for three commercial batches and two batches used for the toxicological tests (Table 1). The average total organic solids (TOS) content of the three commercial batches was 8.7% (w/w); the values ranged from 8.0% to 10.0% (Table 1). The TOS content is a calculated value derived as 100% minus % water minus % ash. The five food enzyme batches presented in Table 1 are concentrates without any added diluents.

The enzyme activity/TOS ratio of the three commercial food enzyme batches ranged from 12.2 to 14.1 KNU(T)/mg TOS (Table 1). The average value of 13.2 KNU(T)/mg TOS was used for subsequent calculations.

Table 1: Compositional data provided for the food enzyme

Parameter	Unit	Batch				
		1	2	3	4[(a)]	5[(b)]
α-Amylase activity	KNU(T)/g[(c)]	976	1,340	1,130	913	1,090
Protein	%	7.2	9.4	7.7	NA	NA
Ash	%	1.2	1.6	1.7	1.6	2.6
Water	%	90.8	88.4	90.3	90.0	86.0
Total organic solids (TOS)[(d)]	%	8.0	10.0	8.0	8.4	11.4
α-amylase activity/mg TOS	KNU(T)/mg TOS	12.2	13.4	14.1	10.9	9.6

(a): Batch used for the genotoxicity tests (Ames and micronucleus).
(b): Batch used for the systemic 90-day oral toxicity study.
(c): KNU(T): Kilo Novo alpha-amylase Units (T standard)/g (see Section 3.1.3).
(d): TOS calculated as 100% - % water - % ash.

The food enzyme complies with the specification for lead (not more than 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 respective limits of detection of the employed methodologies.[4]

No antimicrobial activity was detected in any of these batches (FAO/WHO 2006).

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 per gram.

The applicant has provided information on the identity of the antifoam agents used. Taking into account the nature and properties of the antifoam agents, the manufacturing process and the quality assurance system implemented by the applicant, the Panel considers their use as of no safety concern.

The Panel considered the compositional data provided for the food enzyme as sufficient.

3.1.3. Properties of the food enzyme

The α-amylase catalyses the hydrolysis of α–1,4-glycosidic linkages in starch (amylose and amylopectin), glycogen and related polysaccharides and oligosaccharides, resulting in the generation of soluble dextrins and other oligosaccharides.

The α-amylase activity is quantified by a method based on the hydrolysis of a synthetic substrate (4,6-ethylidene(G7)-*p*-nitrophenyl(G1)-alpha-D-maltoheptaoside (ethylidene-G7pNP)) to glucose and the yellow-coloured *p*-nitrophenol which is determined spectrophotometrically at 405 nm (reaction conditions: 37°C, pH 7.0). The activity is measured relative to an internal enzyme standard, and the result is given in Kilo Novo alpha-amylase Units (T standard)/g (KNU(T)/g).

The temperature/activity profile of the food enzyme was measured from 30°C up to 90°C at pH 4.5 which showed that the α-amylase is active at temperatures up to 85°C with an optimum at 80°C. The pH profile was measured within a pH range from 2.0 to 10.0 at 30°C. The α-amylase is active at pH conditions up to 9.5, with an optimum at pH 7. The α-amylase stability decreases rapidly above 60°C showing no residual activity at 80°C.

[4] Limit of detection (LOD): Pb: 0.5 mg/kg; As: 0.3 mg/kg; Cd and Hg: 0.5 mg/kg.

3.1.4. Information on the source material

3.1.4.1. Information on the genetically modified microorganism

The α–amylase is produced with the genetically modified production strain *B. licheniformis* NZYM-AN, which is deposited in the [redacted] with the deposit number [redacted].

3.1.4.2. Characteristics of the parental and recipient microorganisms

The parental microorganism is the bacterium *B. licheniformis* strain [redacted]. *B. licheniformis* is recommended for the Qualified Presumption of Safety (QPS) status, with the qualification that the absence of acquired antimicrobial resistance genes and toxigenic activity are verified for the specific strain used (EFSA BIOHAZ Panel, 2017a,b).

The parental strain [redacted] was identified as *B. licheniformis* by [redacted]. The strain shows no cytotoxic activity in Chinese hamster ovary cells (Pedersen et al., 2002) and VERO cells. The absence of cytotoxicity has also been shown by the same methodology in strain [redacted], an intermediate strain obtained [redacted] (Pedersen et al., 2002).

The recipient strain *B. licheniformis* [redacted] was derived from [redacted]

3.1.4.3. Characteristics of the introduced sequences

[redacted]

3.1.4.4. Description of the genetic modification process

[redacted]

[redacted]

3.1.4.5. Safety aspects of the production strain

The production strain *B. licheniformis* NZYM-AN differs from the parental strain [redacted]

The stability of the genetic modification was demonstrated by [redacted]

Bacillus licheniformis is recommended for the 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, 2017a,b). The parental strain *B. licheniformis* strain [redacted] and an intermediate strain obtained [redacted] were shown not to be cytotoxic. The absence of acquired antibiotic resistance has not been shown for the production strain, however taking into account the absence of production organism and DNA in the final product (see Section 3.1.5), the Panel did not consider this to be necessary. None of the introduced traits raises safety concerns and therefore the production strain can be presumed to be of no concern.

3.1.5. Manufacturing process

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[5], with food safety procedures based on Hazard Analysis and Critical Control Points (HACCP), and in accordance with Good Manufacturing Practice (GMP).

The food enzyme is produced by a pure culture in a contained, [redacted], [redacted] fermentation system with conventional process controls in place. The identity and purity of the culture are checked at each transfer step from frozen vials until the end of fermentation.

The food enzyme produced is recovered from the fermentation broth after biomass separation using filtration. Further purification and concentration involve a series of filtration steps, including [redacted] and [redacted]. The food enzyme is then formulated as a solid or liquid product.

The absence of the production strain in the product was demonstrated in [redacted]

[5] 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.

[redacted]

No DNA was found in [redacted]

The Panel considered the information provided on the raw materials and the manufacturing process as sufficient.

3.1.6. Safety for the environment

The production strain and its DNA were not detected in the final product. Therefore, the Panel concluded that there is no safety concern for the environment.

3.1.7. Case of need and intended conditions of use

The food enzyme is intended to be used for distilled alcohol production and in starch processing for the production of glucose syrups. The maximum recommended use level of the food enzyme as provided by the applicant is 300 KNU(T)/kg starch dry matter, corresponding to 22.7 mg TOS/kg starch dry matter.

In distilled alcohol production, the food enzyme is added before the slurry mixing step and in the liquefaction step. α-Amylase is intended to be used to convert liquefied starch into a maltose-rich solution, to increase the amounts of fermentable sugars, which results in higher alcohol yields.

In starch processing for the production of glucose syrups, the α-amylase is added during mixing and/or liquefaction, to convert liquefied starch into a maltose-rich solution.

3.1.8. Reaction and fate in food

The α-amylase catalyses the hydrolysis of α−1,4-glycosidic linkages in starch (amylose and amylopectin), glycogen and related polysaccharides and oligosaccharides, resulting in the generation of soluble dextrins and other oligosaccharides.

Experimental data have been provided on the significant removal (> 99%) of protein in the course of distilled alcohol production and starch processing for the production of glucose syrups (Documentation provided to EFSA No 4). The Panel considered this evidence as sufficient to conclude that the residual amounts of TOS (including substances other than proteins) are removed by distillation. In addition, when taking into account the purification steps applied to the production of glucose syrups, i.e. filtration, ion exchange chromatography, treatment with active carbon, the Panel also considered that the amount of TOS (including the substances other than proteins) in the final glucose syrup is removed to a similar degree.

3.2. Dietary exposure

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

3.3. Toxicological data

The Panel considers the production strain to be of no concern on the basis of the reasons stated in Section 3.1.4.5. Moreover, taking into account the intended uses, the exposure is negligible (see Section 3.1.8). Taking this together, the toxicological tests are not needed for the assessment of this food enzyme.

The applicant provided a bacterial gene mutation assay (Ames test), and an *in vitro* mammalian chromosomal aberration test performed with the food enzyme under assessment (batch 4, Table 1). The applicant also provided a repeated dose 90-day oral toxicity study, which has been performed with an α-amylase produced with the derivate of an intermediate upstream strain in the same strain lineage as the strain NZYM-AN (batch 5, Table 1). Despite the view of the Panel that no toxicological tests are needed, the genotoxicity tests were considered as supporting evidence. The repeated dose 90-day oral toxicity study was not considered, as it could not be ascertained that the test item was fully representative of the food enzyme under assessment; however, it is reported in the opinion for completeness.

3.3.1. Genotoxicity

3.3.1.1. Bacterial Reverse Mutation test

A bacterial reverse mutation assay was performed according to OECD Test Guideline 471 (OECD, 1997) and following Good Laboratory Practice (GLP) in four strains of *Salmonella* Typhimurium (TA 1535, TA 1537, TA 98 and TA 100) and *Escherichia coli* WP2uvrA pKM 101, in the presence or absence of metabolic activation (S9-mix) applying a 'treat and plate' assay. Two experiments were carried out using six different concentrations (91, 181, 363, 725, 1,450 and 2,900 μg TOS/mL) of the food enzyme, using appropriate positive controls and deionised water as a negative control. All positive controls showed a distinct increase of induced revertant colonies, confirming the sensitivity of the tests and the efficacy of the S9-mix, while the negative control was within the normal ranges. Upon treatment with the food enzyme, there was no evidence of mutagenic activity at any concentration of the food enzyme in this mutation test. Therefore, the Panel concluded that the food enzyme did not induce gene mutations under the conditions employed.

3.3.1.2. *In vitro* mammalian cell micronucleus test

The *in vitro* micronucleus assay was carried out according to the OECD Test Guideline 487 (OECD, 2010) and following GLP. Human peripheral whole blood cultures obtained from two female donors were exposed to the food enzyme for a short treatment (3 + 21 h) in the presence and absence of S9-mix and a continuous treatment (24 + 24 h) without S9-mix. Proliferation of lymphocytes was stimulated by addition of phytohaemagglutinin to the cultures. The final concentrations scored for micronuclei in this study were 1,000, 2,000 and 3,000 μg TOS/mL in the short-term experiments, as well as 150, 300, 1,000 and 3,000 μg TOS/ml in the long-term treatment experiment. Sterile purified water was used as a solvent, and appropriate positive controls were included. All positive controls induced a statistically significant increase of micronucleus frequency and the system was considered sensitive and valid. Two thousand cells were scored per concentration. In the absence of metabolic activation, highest cytotoxicity based on replication index reduction was 48% after continuous (24 + 24 h) treatment with 3,000 μg TOS/mL. Significant increases in the micronucleated binuclear (MNBN) cell frequency were observed at 1,000 μg TOS/ml following 3 + 21 h treatment with S9-mix ($p \leq 0.05$) and 24 + 24 h treatment without S9-mix ($p \leq 0.01$). However, these effects were not concentration-dependent, not reproducible and fell in the range of historical negative control values. Therefore, they are not considered to be biologically relevant. Treatment of the cells with the test substance resulted in frequencies of MNBN cells, which were similar to and not significantly higher than those observed in concurrent vehicle controls for all the other concentrations analyzed. The Panel concluded that the food enzyme α-amylase did not induce micronuclei in cultured human peripheral blood lymphocytes when tested up to 3,000 μg TOS/mL in the test conditions employed.

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

A repeated dose 90-day oral toxicity study was performed according to OECD test guideline 408 (OECD, 1998), and following GLP. Groups of 10 male and 10 female Sprague–Dawley rats received the food enzyme orally via gavage for 90 days at dose levels of 10%, 33% and 100% food enzyme/kg body weight (bw) per day, adjusted where appropriate with water to dose volumes of 10 mL/kg bw per day, corresponding to 119, 392 and 1,189 mg TOS/kg bw per day (referred to as low-, mid- and high-dose groups respectively). A control group received the tap water alone.

No mortality attributed to intake of the food enzyme was observed. No effects on clinical signs, neurobehavioral changes, body weight, food consumption, ophthalmoscopy, organ weight, macroscopic and microscopic changes were observed that were considered of toxicological relevance.

Statistically significantly lower total water consumption over the study was observed for high-dose males compared to control animals. The finding was considered incidental, as it was only observed in one sex with no dose-relationship.

In mid-dose males, a statistically significantly higher percentage of reticulocytes was observed compared to the control animals before termination of treatment. This was mainly due to a single animal showing a high value and considered to be incidental. In mid-dose males, a statistically significantly lower plasma glucose level was observed before termination of treatment compared to the control animals. Since no dose-dependency was observed and since the data was within historical control data and only observed in one sex, it was considered not to be of toxicological importance.

In mid- and high-dose females, a statistically significantly lower plasma potassium level was observed before termination of treatment compared to the control animals. However, the values were not dose related and within historical control data and were considered not to be of toxicological importance.

The Panel concluded that under the conditions of this repeated dose 90-day oral toxicity study the no observed adverse effect level (NOAEL) was the highest dose tested, which corresponds to 1,189 mg TOS/kg bw per day.

3.4. 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 strain *B. licheniformis* NZYM-AN was assessed by comparison of 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 window of 80 amino acids as the criterion, no match was found.

Limited information is available on occupational respiratory sensitisation to some bacterial α-amylases (Little and Dolovich, 1973; Vanhanen et al., 1997). Fungal α-amylase from *Aspergillus oryzae* is recognised as an occupational respiratory allergen resulting in Baker's asthma (Brisman and Belin, 1991; Sander et al., 1998; Brisman, 2002; Quirce et al., 2002). Despite the wide use of α-amylases, only a low number of case reports of allergic reactions upon oral exposure to α-amylase in individuals sensitised by inhalation to alpha-amylase have been described (Losada et al., 1992; Quirce et al., 1992; Baur and Czuppon, 1995; Kanny and Moneret-Vautrin, 1995; Moreno-Ancillo et al., 2004). Moreover, several studies have shown that adults suffering from occupational asthma due to enzymes used in food (like α-amylase from *A. oryzae*) may be able to ingest the corresponding enzyme without acquiring clinical symptoms of food allergy (Cullinan et al, 1997; Poulsen, 2004; Armentia et al., 2009).

No oral sensitisation and elicitation reactions to the bacterial α-amylase under evaluation have been reported.

The Panel noted that an allergic reaction upon oral ingestion of this α-amylase, produced by the genetically modified *B. licheniformis* strain NZYM-AN, in individuals sensitised to α-amylase cannot be excluded, but the likelihood of such reaction to occur is considered to be low.

The applicant provided a study by Bindslev-Jensen et al. (2006) who investigated the possible cross reactivity of 19 different commercial food enzymes in allergic patients (400 patients allergic to inhalation allergens, food allergens, allergens of bee or wasp). From the three α-amylases tested of different *B. licheniformis* organisms, none was positive in the skin prick test (SPT) or histamine release test. However, as a flare was seen in the area of the wheal in one patient, the SPT was retested with that α-amylase in this patient and found to be negative again. Moreover, this α-amylase was further tested by ingestion (DBPCFC) and found to be negative to both active and placebo challenges. Despite the fact that no allergic reactions have been observed in these individuals, no conclusion can be drawn regarding the enzyme under assessment, since the amino acid sequences of the allergens to which the patients were sensitised are not known.

According to the information provided, substances or products that may cause allergies or intolerances (Regulation (EU) No 1169/2011[6]) are used as raw materials [redacted] in the growth medium of the production organisms. However, during the fermentation process, these products will be degraded and utilised by the bacteria for cell growth, cell maintenance and production of enzyme protein. In addition, the bacterial biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that potentially allergenic residues of these foods employed as protein sources are not expected to be present.

Quantifying the risk for allergenicity is not possible in view of the individual susceptibility to food allergens. Allergenicity can be ruled out only if the proteins are fully removed (e.g. in distilled alcohol

[6] 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.

production). In the starch processing for the production of glucose syrups, although experimental data showed a significant removal (> 99%) of protein, trace amounts of protein, estimated to be up to 0.5 mg/kg, could be present in glucose syrup. Products, such as candy and ice creams, can contain about 50% and 40% glucose syrup, respectively, and therefore, proteins could be present in a quantity sufficient to elicit an allergic reaction.

The Panel considers that under the intended conditions of use the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme cannot be excluded but the likelihood of such reactions to occur is considered to be low.

Conclusions

Based on the microbial source, the genetic modifications, the manufacturing process, the compositional and biochemical data, the removal of TOS during the intended food production processes and the findings in the genotoxicity studies, the Panel concluded that the food enzyme α-amylase produced with the genetically modified *B. licheniformis* strain NZYM-AN does not give rise to safety concerns under the intended conditions of use.

Regarding the allergenicity assessment, 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 can be considered to be low.

Documentation provided to EFSA

1) Dossier 'Application for authorisation of α-amylase produced by a genetically modified strain of *Bacillus licheniformis* (strain NZYM-AN)'. September 2014. Submitted by Novozymes A/S.
2) Summary report on genotoxicity and subchronic toxicity study related to alpha-amylase produced with a strain of *Bacillus licheniformis* (strain NZYM-AN). March 2015. Delivered by FoBiG GmbH, Freiburg (Germany).
3) Summary report on GMM part for alpha-amylase produced by *Bacillus licheniformis* strain NZYM-AN. June 2015. Delivered by DTU, Copenhagen (Denmark).
4) Additional information on 'Food enzyme removal during the production of cereal based distilled alcoholic beverages' and 'Food enzyme carry/over in glucose syrups'. February 2017. Provided by the Association of Manufacturers and Formulators of Enzyme Products.
5) Additional information was received from Novozymes A/S in July 2017.

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Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CFU	colony forming units
DBPCFC	double-blind placebo-controlled food challenge
[redacted]	[redacted]
EC	Enzyme Commission
EINECS	European Inventory of Existing Commercial Chemical Substances ethylidene-G7pNP 4,6-ethylidene(G7)-*p*-nitrophenyl(G1)-alpha-D-maltoheptaoside
FAO	Food and Agriculture Organization
GLP	Good Laboratory Practice
GM	genetically modified
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points
IUBMB	International Union of Biochemistry and Molecular Biology
KNU(T)	Kilo α-amylase units (relative to an internal enzyme standard 'T')
LOD	limit of detection
MNBN	micronucleated binucleated
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-poly acrylamide gel electrophoresis
SPT	skin prick test
TOS	total organic solids
WHO	World Health Organization