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

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Abstract

The food enzyme is an α-amylase (4-α-D-glucan glucanohydrolase; EC 3.2.1.1) produced with the genetically modified *Bacillus licheniformis* strain NZYM-AV by Novozymes A/S. The genetic modifications do not give rise to safety concerns. The food enzyme does not contain the production microorganism or its 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 did not raise a safety concern. The subchronic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rodents. The Panel derived a no observed adverse effect level (NOAEL) at the highest dose level of 796 mg TOS/kg body weight (bw) per day. The allergenicity was evaluated by comparing the amino acid sequence to those of known allergens and one match was found. The Panel considered that, under the intended condition of use, the risk of allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but the likelihood is considered low. Based on the 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 toxicological and 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, α-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:

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;
iii) 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.

Four applications have been submitted by the companies 'Novozymes A/S' and 'AB Enzymes GmbH' for the authorisation of the food enzymes Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-AV), Beta-glucanase, Xylanase and Cellulase produced by a strain of *Humicola insolens* (strain NZYM-ST), Polygalacturonase from a genetically modified strain of *Trichoderma reesei* (strain RF 6197) and Pectin esterase from a genetically modified strain of *Trichoderma reesei* (strain RF 6201).

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] 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.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out safety assessments on the food enzymes Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-AV); Beta-glucanase, Xylanase and Cellulase produced by a strain of *Humicola insolens* (strain NZYM-ST); Polygalacturonase from a genetically modified strain of *Trichoderma reesei* (strain RF 6197) and Pectin esterase from a genetically modified strain of *Trichoderma reesei* (strain RF 6201) 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 α-amylase from a genetically modified strain of *B. licheniformis* strain NZYM-AV.

1.3. Information on existing authorisation and evaluations

The applicant reports that the Danish authority has evaluated and authorised the use of α-amylase produced by genetically modified *B. licheniformis* strain NZYM-AV in starch processing and beverage alcohol (distilling) processes, as well as the conditions of use, including the dosage for specific foods, which were up to a level of 400 Kilo Novo α-amylase Units (KNU(T))/kg starch dry matter.

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 *B. licheniformis* strain NZYM-AV. The food enzyme is intended to be used in several food manufacturing processes: starch processing for the production of glucose syrups, and 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 Guidances from the EFSA Scientific Committee.

The current 'Guidance on the 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 to 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:	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.

3.1.2. Chemical parameters

The α-amylase produced with the genetically modified strain *B. licheniformis* NZYM-AV consists of a single polypeptide of 483 amino acids. The 55.2 kDa molecular mass of the protein was calculated based on the amino acid sequence. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis consistently showed one major protein band in all batches, with an apparent molecular mass of about 48 kDa.

The food enzyme was tested for other enzyme activities. Glucoamylase, lipase and protease activities were below the limits of detection (LOD) of the methods applied.[4]

Data on the chemical parameters of the food enzyme have been provided for three batches used for commercialisation (1, 2 and 3) and one batch (4) used for toxicological testing (Table 1). The average total organic solids (TOS) of the three commercial batches was 3.5% (w/w); the values ranged from 3.0% to 4.0%.

The enzyme activity/TOS ratio of the three commercial food enzyme batches ranged from 14.9 to 20.7 KNU(T)/mg TOS (Table 1). The average value of 18.7 KNU(T)/mg TOS was used for subsequent calculations.

Table 1: Compositional data provided for the food enzyme

Parameter	Units	Batch			
		1	2	3	4[(a)]
α-Amylase activity	KNU(T)/g batch[(b)]	612	705	594	710
Protein	%	2.8	3.3	2.7	2.8
Ash	%	0.6	0.5	0.4	3.4
Water	%	96.4	96.1	95.6	89.0
Total organic solids (TOS)[(c)]	%	3.0	3.4	4.0	7.6
α-Amylase activity/mg TOS	KNU(T)/mg TOS	20.4	20.7	14.9	9.3

(a): Batch used for the toxicological studies.
(b): KNU(T): Kilo Novo α-amylase Units (relative to an internal enzyme standard 'T') (see Section 3.1.3).
(c): 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 their respective limits of detection of the employed methodologies.[5]

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 should not exceed 30 colony forming units (CFU) per gram.

The applicant has provided information on the identities 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 analytical principle to determine the α-amylase activity is based on hydrolysis of the substrate 4,6-ethylidene(G7)-*p*-nitrophenyl(G1)-α-D-maltoheptaoside to glucose and *p*-nitrophenol (pH 7.2, 37°C, 5 min). *p*-Nitrophenol is measured spectrophotometrically at 405 nm. The activity is measured relative to an internal enzyme standard and expressed in KNU(T)/g.

The food enzyme has been characterised regarding its temperature and pH profiles. The α-amylase has a temperature optimum around 80°C (pH 4.5) and a pH optimum around 8.0 (T 30°C). Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures and showed that the activity is retained at temperatures up to 50°C. Under the conditions (pH 3.0) of the applied temperature stability assay, the α-amylase activity decreased rapidly above 50°C showing no residual activity above 75°C.

[4] LODs: glucoamylase = 0.83 AGU/g; lipase = 0.02 KLU/g; protease = 196 HUT/g.
[5] LODs: Pb = 0.5 mg/kg; As = 0.1 mg/kg; Cd = 0.05 mg/kg; Hg = 0.03 mg/kg.

3.1.4. Information on the source material

3.1.4.1. Information related to the genetically modified microorganism

The α-amylase is produced with the genetically modified strain *B. licheniformis* NZYM-AV, which is deposited at the [redacted] with 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). 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] The resulting strain was the production strain NZYM-AV.

[redacted]

All integration sites were sequenced and the correct insertions documented. [redacted]

3.1.4.5. Safety aspects of the production strain

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

The genetic stability of the production strain was demonstrated [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 [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 raise 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[6], 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] 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]. After stabilisation with [redacted], the food enzyme preparation is commercialised in a liquid or in a solid form.

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

[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.

[redacted]

A test for DNA in the food enzyme was made [redacted]. No DNA was detected [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 environment.

3.1.7. Case of need and intended conditions of use

The food enzyme is intended to be used for distilled alcohol production processes and starch processing for the production of glucose syrups. The maximum recommended use level of the food enzyme as provided by the applicant is 400 KNU(T)/kg starch, corresponding to 21.4 mg TOS/kg of starch.

In distilled alcohol production, the food enzyme is added during 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 processes, the α-amylase is added during mixing and/or liquefaction and/or saccharification of starch.

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 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 the evidence as sufficient to conclude that 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 considers that the amount of TOS (including 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 to the food enzyme is negligible (see Section 3.1.8). Taking this together, the toxicological tests are not needed for the assessment of this food enzyme.

A battery of toxicological tests including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian chromosomal aberration test, and a repeated dose 90-day oral toxicity study in rats has been provided. Despite the view of the Panel that no toxicological tests are needed, the tests were considered as supporting evidence. The batch 4 (Table 1) which is used for toxicological testing has lower specific activity compared to the batches used for commercialisation, and thus is considered cruder and suitable for toxicological testing.

3.3.1. Genotoxicity

3.3.1.1. Bacterial reverse mutation test

To investigate the potential of the α-amylase to induce gene mutations, 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) in four strains of *Salmonella* Typhimurium (TA 1535, TA 1537, TA 98 and TA 100) and *E. coli* WP2uvrApKM 101, in the presence or absence of metabolic activation applying the 'treat and plate' assay. Two experiments were carried out using six different concentrations (156, 313, 625, 1,250, 2,500 and 5,000 μg dry matter/mL, corresponding to 108, 216, 432, 864, 1,727, and 3,455 μg TOS/mL) of the food enzyme using appropriate positive control chemicals and water as a negative control. All positive controls induced a significant increase in the number of revertant colonies, confirming the sensitivity of the assay and the efficacy of the S9-mix. Growth stimulation was observed in some tested conditions after treatment with the food enzyme; the increase did not result in an increase in the corresponding number of revertant colonies above the control values in any strain with or without S9-mix.

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

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. Whole blood cultures were treated with the food enzyme, positive controls and purified water (negative control) both in the presence and absence of metabolic activation. Based on the results of a preliminary cytotoxicity assay, the cells were treated with 3,000, 4,000 and 5,000 μg food enzyme/mL (corresponding to 228, 308 and 380 μg TOS/mL) applying a short-term treatment (3 + 21 h of recovery) in the presence and absence of S9-mix, and with 1,000, 3,000 and 5,000 μg food enzyme/mL (corresponding to 76, 228 and 380 μg TOS/mL) applying a continuous treatment (24 + 24 h of recovery) without S9-mix. Two thousand cells were scored per experimental point. The positive controls induced statistically significant increases in micronucleus (MN) frequency and the system was considered sensitive and valid. Negative controls were within the historical vehicle control ranges. Cytotoxicity, measured as mitotic inhibition, did not exceed 22% relative to the concurrent vehicle controls. No increase in the number of micronucleated binucleated (MNBN) cells was observed after short-term treatment with the food enzyme both in the presence and absence of metabolic activation. A statistically significant increase in MNBN cell frequency was observed at 5,000 μg food enzyme/mL after continuous treatment without S9-mix (0.3% vs 0.93% MNBN cells at 0 and 5,000 μg/mL, respectively). Four slides were scored from this concentration (1,000 binucleated cells per slide). The Panel noted that the increase was within the 95% of the reference range for the historical negative controls and that only one out of four replicate cultures showed a MNBN cell frequency that exceeded the historical vehicle range. Therefore, the observed increase was considered not biologically relevant.

The Panel concluded that the food enzyme α-amylase did not induce MN under the test conditions employed for this study.

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 via gavage the food enzyme at a volume of 10 mL/kg body weight (bw) per day corresponding to 80, 263 and 796 mg TOS/kg bw per day (referred to as low-, mid- and high-dose groups, respectively). Controls received the vehicle (water) alone.

No mortality was observed.

The group mean grip strength for high-dose males was statistically significantly increased. In the mid-and high-dose females, a statistically significantly low rearing activity (high beam scores) was observed during the initial 6-min interval without a dose–response relationship, and which continued for the high-dose females during the second 6-min interval. Because of the isolated nature of these slight but statistically significant differences, and the increased or reduced values in the historical control data, they were considered incidental and reflected normal variation.

The weight gains of the mid-dose males and females were statistically significantly increased (approximately to 108% and 112% of control values for males and females, respectively). However, in the absence of a similar finding in the high-dose animals, this effect was not considered of toxicological relevance.

In haematology, a statistically significantly increased prothrombin time was observed for the mid- and high-dose males. A statistically significantly increased large unstained cell (LUC) count was observed in the mid-dose males. As these changes were minor, they were considered of no toxicological importance.

No other significant effects were observed.

The Panel concluded that under the conditions of this repeated dose 90-day oral toxicity study the NOAEL was the highest dose tested, which corresponds to 796 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-AV 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 micro-organisms 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, one match was found. The matching allergen is Asp o 21, an α-amylase produced by *Aspergillus oryzae* known as an occupational respiratory allergen.

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 *A. 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 α-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, as described for 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 with the genetically modified *B. licheniformis* strain NZYM-AV, in individuals sensitised to α-amylase cannot be excluded, but the likelihood of such a 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 (double-blind, placebo-controlled food challenge) 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[7]) are used as raw materials ([redacted]) in the growth medium of the production organism. 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

[7] 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 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 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 quantities sufficient to elicit an allergic reaction.

The Panel considers that under the intended conditions of use the risk of allergic sensitisation and elicitation reactions by 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 toxicological studies, the Panel concluded that the food enzyme α-amylase produced with the genetically modified *B. licheniformis* strain NZYM-AV 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 'Alpha-amylase produced by a genetically modified strain of *Bacillus licheniformis* (strain NZYM-AV)'. August 2014. Submitted by Novozymes A/S (Denmark).
2) Summary report on genotoxicity and subchronic toxicity study related to alpha-amylase produced with a strain of Bacillus licheniformis (strain NZYM-AV). June 2015. Delivered by FoBiG GmbH (Freiburg, Germany).
3) Summary report on GMM part for alpha-amylase produced by *Bacillus licheniformis* strain NZYM-AV. February 2016. Delivered by the Technical University of Denmark (Søborg, 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 January 2018.

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Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids
CFU	colony forming units
[redacted]	[redacted]
EC	Enzyme Commission
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization
GLP	Good laboratory practice
GM	genetically modified
GMO	genetically modified organism
GMP	Good manufacturing practice
HACCP	Hazard analysis and critical control points
IUBMB	International Union of Biochemistry and Molecular Biology
KNU(T)	Kilo Novo α-amylase units (relative to an internal enzyme standard 'T')
LOD	limit of detection
LUC	large unstained cell
MN	micronucleus
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