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Safety evaluation of the food enzyme glucan 1,4- α -glucosidase from a genetically modified *Aspergillus niger* (strain NZYM-BW)

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Abstract

The food enzyme glucan 1,4- α -glucosidase (EC 3.2.1.3) is produced with the genetically modified *Aspergillus niger* strain NZYM-BW 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 recombinant DNA. The glucan 1,4- α -glucosidase food enzyme is intended to be used in distilled alcohol production and starch processing for the production of glucose syrups. 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 systemic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rodents. The Panel identified a no observed adverse effect level at the highest dose of 1,244 mg TOS/kg body weight (bw) per day. Similarity of the amino acid sequence to those of known allergens was searched and one match was found. The Panel considered that, under the intended condition of use, the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme cannot be excluded, but the likelihood of such reactions occurring is considered to be low. Based on the data provided and the removal of TOS during the intended food production processes, the Panel concluded that this food enzyme does not raise safety concerns under the intended conditions of use.

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Keywords: food enzyme, glucoamylase, glucan 1,4- α -glucosidase, EC 3.2.1.3, *Aspergillus niger*, genetically modified microorganism

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1. Introduction

Article 3 of the Regulation (EC) No 1332/2008¹ 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 microorganisms: (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 1331/2008² 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 an approval via an EU Community list.

The 'Guidance on submission of a dossier on a food enzyme for 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 1332/2008¹ on food enzymes.

Five applications have been introduced by the companies "Novozymes A/S", DSM Food Specialities B.V., "Advanced Enzyme Technologies Ltd" and the "Association of Manufacturers and Formulators of Enzyme Products (AMFEP)" for the authorisation of the food enzymes Pullulanase from a genetically modified strain of *Bacillus subtilis* (strain NZYM-AK), Glucoamylase from a genetically modified strain of *Aspergillus niger* (strain NZYM-BW), Chymosin from a genetically modified strain of *Kluyveromyces lactis* (strain CHY), Pectin lyase from a genetically modified strain of *Aspergillus niger* (strain FLOSC) and Triacylglycerol lipase from pregastric tissues of cattle, goat and sheep respectively.

Following the requirements of Article 12.1 of Commission Regulation (EU) No 234/2011³ implementing Regulation (EC) No 1331/2008², 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, p. 7–15.

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

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

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes Pullulanase from a genetically modified strain of *Bacillus subtilis* (strain NZYM-AK), Glucoamylase from a genetically modified strain of *Aspergillus niger* (strain NZYM-BW), Chymosin from a genetically modified strain of *Kluyveromyces lactis* (strain CHY), Pectin lyase from a genetically modified strain of *Aspergillus niger* (strain FLOSC) and Triacylglycerol lipase from pregastric tissues of cattle, goat and sheep in accordance with the article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission request to carry out the safety assessment of the food enzyme glucoamylase from *A. niger* strain NZYM-BW submitted by Novozymes A/S.

1.3. Information on existing authorisations and evaluations

The applicant indicates that the food enzyme object of the present dossier has not been evaluated by authorities in the EU. The applicant also reports that the Brazilian, Canadian, Chinese, Danish, French, Mexican, Russian and South Korean authorities have evaluated and authorised the use of glucoamylases and/or glucoamylases from genetically modified strains of *A. niger*.

2. Data and methodologies

2.1. Data

The applicant submitted a dossier supporting the application for authorisation of the food enzyme glucan 1,4- α -glucosidase produced with a genetically modified microorganism (GMM), *A. niger* (strain NZYM-BW). The food enzyme is intended to be used in distilled alcohol production and starch processing for the production of glucose syrups.

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 the submission of a dossier for safety evaluation of a food enzyme (EFSA, 2009) has been followed for the evaluation of this 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:	Glucan 1,4- α -glucosidase
Systematic name:	4- α -D-glucan α -glucohydrolase
Synonyms:	Glucoamylase
IUBMB No:	EC 3.2.1.3
CAS No:	9032-08-0
EINECS No.:	232-877-2

Glucan 1,4- α -glucosidases catalyse the hydrolysis of terminal 1,4- α linkages in the starch polysaccharides, amylose and amylopectin, and their hydrolysis' products (dextrins) releasing free D-glucose. They also catalyse the hydrolysis of 1,6- α linkages in amylopectin, but at a slower rate. It is intended to be used in distilled alcohol production and starch processing for the production of glucose syrups.

3.1. Source of the food enzyme

The glucan 1,4- α -glucosidase is produced with a GM filamentous fungus *A. niger*. The technical dossier contains all necessary information on the recipient microorganism, the donor organism and the genetic modification process.

The production strain *A. niger* NZYM-BW is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) with accession number [REDACTED].⁴

3.1.1. Characteristics of the parental and recipient microorganisms⁵

The parental strain is *A. niger* [REDACTED]. The first intermediate strain [REDACTED] is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) with accession number [REDACTED]. It was derived from the parental strain by [REDACTED]. Strain [REDACTED] was identified as *A. niger* by [REDACTED].

Recipient strain *A. niger*

[REDACTED]

An isolate with improved glucoamylase production was identified
During the development of the recipient strain [REDACTED]

[REDACTED]

3.1.2. Characteristics of the introduced sequences

The sequence encoding the [REDACTED]

[REDACTED]

3.1.3. Description of the genetic modification process

The purpose of genetic modification was to enable the production strain to synthesise glucan 1,4- α -glucosidase from [REDACTED]

[REDACTED]

The recipient strain [REDACTED]

[REDACTED]

⁴ Technical dossier: Annex A4.

⁵ Technical dossier: Annex 4.

3.1.4. Safety aspects of the genetic modification

The production strain *A. niger* NZYM-BW differs from the recipient strain [REDACTED] only in its capacity to produce the glucan 1,4- α -glucosidase enzyme from [REDACTED]. The presence and the location of the enzyme encoding gene were confirmed by Southern analysis. The phenotypic stability of the *A. niger* NZYM-BW strain was confirmed by its capacity to produce a constant level of the enzyme glucan 1,4- α -glucosidase measured in relation to the TOS in three independent batches of the food enzyme and its genetic stability was demonstrated [REDACTED].

The absence of the antibiotic resistance genes used during the genetic modification was confirmed by Southern analysis of the production strain NZYM-BW with probes specific to the [REDACTED] resistance genes. The absence of the [REDACTED] was also confirmed by Southern analysis.⁶

No issues of concern arising from the genetic modifications were identified by the Panel.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004⁷, with food safety procedures based on Hazard Analysis and Critical Control Points (HACCP), and in accordance with current Good Manufacturing Practice (GMP).

The production strain is grown as a pure culture using a typical industrial medium in a submerged, fed-batch fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration leaving a supernatant containing the food enzyme. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in which enzyme protein is retained while low molecular weight material passes the filtration membrane and is discarded. The applicant provided information on the identity of the substances used to control the fermentation^{8,9} and in the subsequent downstream processing of the food enzyme.

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

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The glucan 1,4- α -glucosidase is a single polypeptide chain of 558 amino acids.¹⁰ The molecular mass of the mature protein derived from the amino acid sequence (without the signal peptide) was calculated to be 59.2 kDa. The protein pattern of the food enzyme was investigated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis. Gels showed a major band with an apparent molecular mass of about 60 kDa which is consistent with the calculated value for the glucan 1,4- α -glucosidase.¹¹ The food enzyme concentrate was tested for α -amylase, lipase and protease activities, which were below the limits of detection of the methods applied.¹² No other enzymatic side activities were reported.

The in-house determination of glucan 1,4- α -glucosidase activity is based on hydrolysis of the substrate maltose (reaction conditions: pH 4.3, temperature 37°C, reaction time 6 min). The enzymatic activity is determined by measuring the release of glucose. The glucan 1,4- α -glucosidase activity is quantified relative to an enzyme standard and expressed in Amyloglucosidase Units/g (AGU(D)/g).

⁶ Technical dossier: Annex D1.

⁷ Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of food additives. OJ L 226, 25.6.2004, p. 3–21.

⁸ Technical dossier: Annex 6.

⁹ Technical dossier: Annex 2.07.

¹⁰ Technical dossier: p. 31 and Annex 1.

¹¹ Technical dossier: p. 33.

¹² Technical dossier: p. 41 and annexes: 3.02, 3.03, 3.04; Technical dossier p. 60 (tox batch).

The food enzyme has been characterised with regard to its temperature and pH profiles. It has a temperature optimum around 70°C (pH 4.3) and a pH optimum around pH 3.5 (temperature 37°C). Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures. Under the conditions (pH 4.3) of the applied temperature stability assay, the glucan 1,4- α -glucosidase activity decreased rapidly above 60°C showing no residual activity after pre-incubation at temperatures above 75°C.

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for four food enzyme batches, three batches used for commercialisation and one batch produced for the toxicological tests (Table 1). The average total organic solids (TOS) of the three food enzyme batches for commercialisation was 11.3% (range 11.2–11.4%). The average enzyme activity/TOS ratio of the three food enzyme batches for commercialisation is 5.1.

Table 1: Compositional data of the food enzyme

Parameter	Unit	Batch			
		1	2	3	4 ^(a)
Glucan 1,4- α -glucosidase activity	AGU(D)/g batch ^(b)	553	582	597	454
Protein	%	9.4	10.2	10.3	NA ^(d)
Ash	%	0.1	0.5	0.2	1.2
Water	%	88.5	88.1	88.6	86.9
Total organic solids (TOS) ^(c)	%	11.4	11.2	11.2	11.9
Activity/mg TOS	AGU(D)/mg TOS	4.8	5.1	5.3	3.8

(a): Batch used for the toxicological studies.

(b): AGU(D): Amyloglucosidase Units (see Section 3.1.3).

(c): TOS calculated as 100% - % water - % ash.

(d): NA: not analysed.

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).¹³

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 *E. coli* and *Salmonella* species are absent in 25 g of sample and total coliforms should not exceed 30 colony forming unit (CFU) per gram. No antimicrobial activity was detected in any of these batches (FAO/WHO, 2006).¹⁴

Mycotoxins, particularly ochratoxin A and fumonisins, are produced by many strains of *A. niger* (Blumenthal, 2004; Frisvad et al., 2007, 2011; EFSA BIOHAZ Panel, 2017).

the strain unable to produce ochratoxin A and fumonisins. This was confirmed by analysis of the four batches of food enzyme in which the levels of these mycotoxins were found to be below the limits of detection.

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 the production strain in the food enzyme was demonstrated in three independent batches analysed in triplicate.

¹³ Additional information January 2018.

¹⁴ Technical dossier: pp. 34 and 60 (tox batch).

A test for recombinant DNA in the food enzyme was made by polymerase chain reaction (PCR) analysis of three batches in triplicate. [REDACTED]

The production strain and recombinant DNA could not be detected in samples from three fermentation batches.

3.4. Toxicological data

The food enzyme batch 4 used for the toxicological assays has a lower activity/mg TOS than the three batches for commercialisation (Table 1). This indicates a slightly lower purity than the commercial batches and thus batch 4 was considered suitable for the toxicological testing.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test¹⁵

A bacterial reverse mutation assay (Ames test) was made according to OECD Test Guideline 471 (OECD, 1997) and following Good Laboratory Practice (GLP). 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 using six different concentrations of the food enzyme (156, 313, 625, 1,250, 2,500 and 5,000 μg dry matter/mL, corresponding to 142, 284, 568, 1,135, 2,271 and 4,542 μg TOS/mL), and appropriate positive and negative controls. Toxic effects, evident as a reduction in the number of revertants, occurred in TA98 in the absence of S9-mix at 2,500 $\mu\text{g}/\text{mL}$ and 5,000 $\mu\text{g}/\text{mL}$ in the second experiment. Growth stimulation, as demonstrated by increases in the viable 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 there was no significant increase in revertant colony numbers above the control values in any strain with or without S9-mix.

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

3.4.1.2. *In vitro* micronucleus assay¹⁶

The *in vitro* micronucleus test was carried out according to OECD Draft Guideline 487 (OECD, 2010) and following GLP. Two separate experiments were performed in duplicate cultures of human peripheral whole blood lymphocytes treated with deionised water (negative control), the food enzyme or appropriate positive controls. Cells were exposed to the test substance for 3 h 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 h after removal of the test substance (24 + 24 h treatment). The food enzyme was tested at 3,000, 4,000 and 5,000 μg food enzyme/mL, corresponding to 357, 476 and 595 μg TOS/mL. No cytotoxicity was observed after treatments, both in the presence and absence of S9-mix. The frequency of micronuclei was comparable to the negative controls at all doses tested; exception was the 3 + 21 h treatment in the presence of S9-mix at 5,000 $\mu\text{g}/\text{mL}$ where a statistically significant increase of micronucleated binucleate cells was observed. The Panel noted that these values were within the 95% of the historical control range.

The Panel concluded that the food enzyme glucan 1,4- α -glucosidase did not induce micronuclei 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 rodents¹⁷

The repeated dose 90-day oral toxicity study was performed in accordance with OECD Test Guideline 408 (OECD, 1998) and following GLP. Groups of 10 male and 10 female Sprague–Dawley (CrI:CD(SD)) rats received by gavage the food enzyme corresponding to 124, 410, or 1,244 mg TOS/kg body weight (bw) per day. Controls received the vehicle (water).

No mortality was observed. The functional observation battery tests (cage floor and rearing activity) showed that high-dose males had significantly decreased low (1/10) and high (2/10) beam scores

¹⁵ Technical dossier: Annex 7.01.

¹⁶ Technical dossier: Annex 7.02.

¹⁷ Technical dossier: Annex 7.03.

compared with concurrent controls. There were no similar findings in females. Overall these observations were considered incidental.

In haematology slightly, but significantly increased erythrocyte counts in mid- and high-dose males were observed. Since there was no clear dose-response and no changes in other erythrocytic indices, the effects were considered incidental and not treatment-related. A slightly but significantly increased large unstained cell count in high-dose males was also observed. This was attributed only to two animals with values above the background range and not considered of toxicological concern. In blood chemistry, a slightly but significantly decreased creatinine concentration in high-dose males was observed. In addition, the potassium concentration was increased in high-dose males and decreased in high-dose females. These changes were considered of no toxicological importance.

Urinalysis revealed a dose-related reduction in pH for males, reaching significance in the mid- and high-dose groups. The alterations were within the range of the historical control data and lacked consistency between sexes. There were no histopathological changes in the kidneys which could be considered toxicologically relevant.

No other significant effects were observed. Overall, the Panel identified a no observed adverse effect level (NOAEL) at the high-dose level of 1,244 mg TOS/kg bw per day.

3.4.3. Allergenicity¹⁸

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 glucan 1,4- α -glucosidase produced with the GM *A. niger* strain NZYM-BW was assessed by comparison of its amino acid sequence with those of known allergens according to the EFSA 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 criterion, one match was found. The matching allergen was Sch c 1, a glucoamylase from *Schizophyllum commune*, an enzyme described as an occupational respiratory allergen associated with baker's asthma (Quirce et al., 2002; Toyotome et al., 2014).

No information is available on oral sensitisation or elicitation reactions of this glucan 1,4- α -glucosidase. Several studies have shown that adults with occupational asthma may be able to ingest respiratory allergens without acquiring clinical symptoms of food allergy (Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). In addition, no allergic reactions upon dietary exposure to any glucoamylase have been reported in the literature. Therefore, it can be concluded that the likelihood of an allergic reaction upon oral ingestion of this glucan 1,4- α -glucosidase, produced with the GM *A. niger* strain NZYM-BW, in individuals respiratory sensitised to glucoamylase, cannot be excluded, but the likelihood of such a reaction to occur is considered to be low.

According to the information provided, substances or products that may cause allergies or intolerances (Regulation EU 1169/2011)¹⁹ are used as raw materials in the growth medium of the production organism. However, during the fermentation process, these products will be degraded and utilised by the fungus for cell growth, cell maintenance and production of enzyme protein. In addition, the fungal biomass and fermentation solids will be removed. Considering 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. 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.

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

¹⁸ Technical dossier: Annex 8.

¹⁹ 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.

3.5. Dietary exposure

The glucan 1,4- α -glucosidase is intended to be used in distilled alcohol production and starch processing for the production of glucose syrups at an intended use level of up to 800 AGU(D)/kg starch dry matter.

In starch processing, maltodextrins are degraded in the saccharification step by glucoamylases. Thus, the food enzyme contributes to efficient degradation of dextrans and production of glucose with a reduced risk of microbial contamination because it can be used at high operating temperature. Similarly, in beverage alcohol (distilling) processing, the food enzyme contributes to efficient degradation of dextrans and production of fermentable sugars, resulting in high alcohol yields due to a more complete conversion of starch.

Experimental data have been provided showing 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 3). 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, 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 in the final glucose syrup will be removed to a similar degree.

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.

4. Conclusions

Based on the data provided and on the removal of TOS during the intended food production processes, the Panel concluded that the food enzyme glucan 1,4- α -glucosidase produced with the genetically modified *A. niger* strain NZYM-BW 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.

Documentation provided to EFSA

- 1) Dossier "Glucoamylase produced by a genetically modified strain of *Aspergillus niger* (strain NZYM-BW)". March 2015. Submitted by Novozymes A/S.
- 2) Additional information was received from Novozymes A/S in January 2018.
- 3) 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.

References

- Armentia A, Díaz-Perales A, Castrodeza J, Dueñas-Laita A, Palacin A and Fernández S, 2009. Why can patients with baker's asthma tolerate wheat flour ingestion? Is wheat pollen allergy relevant? *Allergologia et Immunopathologia*, 37, 203–204.
- Blumenthal CZ, 2004. Production of toxic metabolites in *Aspergillus niger*, *Aspergillus oryzae*, and *Trichoderma reesei*: justification of mycotoxin testing in food grade enzyme preparations derived from the three fungi. *Regulatory Toxicology and Pharmacology*, 39, 214–228.
- Brisman J, 2002. Baker's asthma. *Occupational and Environmental Medicine*, 59, 498–502; quiz 502, 426.
- EFSA (European Food Safety Authority), 2009. Guidance of the Scientific Committee on Transparency in the Scientific Aspects of Risk Assessments carried out by EFSA. Part 2: general Principles. *EFSA Journal* 2009;7(5):1051, 22 pp. <https://doi.org/10.2903/j.efsa.2009.1051>
- EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Ricci A, Allende A, Bolton D, Chemaly M, Davies R, Girones R, Herman L, Koutsoumanis K, Lindqvist R, Nørrung B, Robertson L, Ru G, Sanaa M, Simmons M, Skandamis P, Snary E, Speybroeck N, Ter Kuile B, Threlfall J, Wahlström H, Cocconcelli PS, Klein G (deceased), Prieto Maradona M, Querol A, Peixe L, Suarez JE, Sundh I, Vlak JM, Aguilera-Gomez M, Barizzone F, Brozzi R, Correia S, Heng L, Istace F, Lythgo C and Fernández Escámez PS, 2017. Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA. *EFSA Journal* 2017;15(3):4664, 177. <https://doi.org/10.2903/j.efsa.2017.4664>

- EFSA CEF Panel (EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids), 2009. Guidance of the Scientific Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) on the Submission of a Dossier on Food Enzymes for Safety Evaluation by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids. EFSA Journal 2009;7(8):1305, 1–26. <https://doi.org/10.2903/j.efsa.2009.1305>
- EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), Silano V, Bolognesi C, Castle L, Cravedi J-P, Fowler P, Franz R, Grob K, Gürtler R, Husøy T, Karenlampi S, Mennes W, Milana MR, Penninks A, Smith A, Tavares Pocas MF, Tlustos C, Wolffe D, Zorn H, Zugravu C-A, Arcella D, Liu Y and Engel K-H, 2016. Panel statement on the exposure assessment of food enzymes. EFSA Journal 2016;14(11):4581, 9 pp. <https://doi.org/10.2903/j.efsa.2016.4581>
- EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010. Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal 2010;8(7):1700, 168 pp. <https://doi.org/10.2903/j.efsa.2010.1700>
- FAO/WHO (Food and Agriculture Organization of the United States/World Health Organization), 2006. General specifications and considerations for enzyme preparations used in food processing in Compendium of food additive specifications. 67th meeting. FAO JECFA Monographs 3, pp. 63–67. Available online: <ftp://ftp.fao.org/docrep/fao/009/a0675e/a0675e00.pdf>
- Frisvad JC, Smedsgaard J, Samson RA, Larsen TO and Thrane U, 2007. Fumonisin B2 production by *Aspergillus niger*. Journal of Agricultural and Food Chemistry, 55, 9727–9732.
- Frisvad JC, Larsen TO, Thrane U, Meijer M, Varga J, Samson RA and Nielsen KF, 2011. Fumonisin and ochratoxin production in industrial *Aspergillus niger* strains. PLoS ONE, 6, e23496. <https://doi.org/10.1371/journal.pone.0023496>
- OECD (Organisation for Economic Co-operation and Development), 1997. Bacterial Reverse Mutation Test. Guideline 471, adopted 21.07.1997. Available online: http://www.oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en;jsessionid=9zfgzu35paaq.x-oecd-live-01
- OECD (Organisation for Economic Co-operation and Development), 1998. Repeated Dose 90-day Oral Toxicity Study in Rodents. Guideline 408, adopted 21.09.1998. Available online: http://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en
- OECD (Organisation for Economic Co-operation and Development), 2010. Genetic Toxicology: OECD Guideline for the testing of chemicals. Guideline 487, adopted 22.07.2010. Draft proposal for a new Guideline 487: *In vitro* Micronucleus Test. Available online: http://www.oecd-ilibrary.org/environment/test-no-487-in-vitro-mammalian-cell-micronucleus-test_9789264091016-en

- Poulsen LK, 2004. Allergy assessment of foods or ingredients derived from biotechnology, gene-modified organisms, or novel food. Molecular Nutrition & Food Research, 48, 413–423.
- Quirce S, Fernandez-Nieto M, Bartolome B, Bombin C, Cuevas M, Sastre J, 2002. Glucoamylase: another fungal enzyme associated with baker's asthma. Annals of Allergy Asthma & Immunology, 89, 197–202.
- Toyotome T, Satoh M, Yahiro M, Watanabe A, Nomura F and Kamei K, 2014. Glucoamylase is a major allergen of *Schizophyllum commune*. Clinical & Experimental Allergy, 44, 450–457. <https://doi.org/10.1111/cea.12260>

Abbreviations

AGU(D)	Amyloglucosidase Units
AMFEP	Association of Manufacturers and Formulators of Enzyme Product
bw	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
CFU	colony forming units
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization
FUM	fumonisin
GLP	Good Laboratory Practice
GM	genetically modified
GMM	genetically modified microorganism
GMO	genetically modified organisms
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points

ITS	Internal transcribed spacer (sequencing)
IUBMB	International Union of Biochemistry and Molecular Biology
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
OTA	ochratoxin A
PCR	polymerase chain reaction
TOS	total organic solids
WHO	World Health Organization