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## Safety evaluation of the food enzyme glucan 1,4- $\alpha$ -glucosidase from the genetically modified *Aspergillus niger* strain NZYM-BR

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### Abstract

The food enzyme glucan 1,4- $\alpha$ -glucosidase (4- $\alpha$ -D-glucan glucohydrolase, EC 3.2.1.3) is produced with the genetically modified *Aspergillus niger* strain NZYM-BR by Novozymes A/S. The genetic modifications do not give rise to safety concerns. The food enzyme is free from viable cells of the production organism and its DNA. The food enzyme is intended to be used in starch processing for the production of glucose syrups and distilled alcohol. Since residual amounts of total organic solids (TOS) are removed by the purification steps applied during the production of glucose syrups and by distillation, dietary exposure estimation was considered not necessary. 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 rats. The Panel identified a no observed adverse effect level of 1,135 mg TOS/kg body weight (bw) per day, the highest dose tested. Similarity of the amino acid sequence of the food enzyme to those of known allergens was searched for and two matches were found. The Panel considered that under the intended conditions of use (other than distilled alcohol production) the risk of allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but the likelihood for this to occur is considered to be low. Based on the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

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**Keywords:** food enzyme, glucan 1,4- $\alpha$ -glucosidase, 4- $\alpha$ -D-glucan glucohydrolase, EC 3.2.1.3, *Aspergillus niger*, genetically modified microorganism

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## 1. Introduction

Article 3 of the Regulation (EC) No 1332/2008<sup>1</sup> provides definition for 'food enzyme' and 'food enzyme preparation'.

'Food enzyme' means a product obtained from plants, animals or micro-organisms or products thereof including a product obtained by a fermentation process using micro-organisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.

'Food enzyme preparation' means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

Before January 2009, food enzymes other than those used as food additives were not regulated or were regulated as processing aids under the legislation of the Member States. On 20 January 2009, Regulation (EC) No 1332/2008 on food enzymes came into force. This Regulation applies to enzymes that are added to food to perform a technological function in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food, including enzymes used as processing aids. Regulation (EC) No 1331/2008<sup>2</sup> established the European Union (EU) procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

- it does not pose a safety concern to the health of the consumer at the level of use proposed;
- there is a reasonable technological need;
- its use does not mislead the consumer.

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

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

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

#### 1.1.1. Background as provided by the European Commission

Only food enzymes included in the European Union (EU) Community list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7 (2) of Regulation (EC) No 1332/2008 on food enzymes.

Three applications have been introduced by the company Novozymes A/S for the authorisation of the food enzymes alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-BC), amyloglucosidase from a genetically modified strain of *Aspergillus niger* (strain NZYM-BR) and glucose oxidase from a genetically modified strain of *Aspergillus oryzae* (strain NZYM-KP).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011<sup>3</sup> implementing Regulation (EC) No 1331/2008, the Commission has verified that the three 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 the safety assessments on the food enzymes alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-BC), amyloglucosidase from a genetically modified strain of *Aspergillus niger*

<sup>1</sup> 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.

<sup>2</sup> 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.

<sup>3</sup> 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.

(strain NZYM-BR) and glucose oxidase from a genetically modified strain of *Aspergillus oryzae* (strain NZYM-KP) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

## 1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of the food enzyme amyloglucosidase (synonymous for glucan 1,4- $\alpha$ -glucosidase) from the genetically modified strain of *A. niger* (strain NZYM-BR).

## 2. Data and methodologies

### 2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme amyloglucosidase from a genetically modified strain of *A. niger* (strain NZYM-BR). The dossier was updated on 5 November 2020.

Additional information was requested from the applicant during the assessment process on 19 March 2015, 29 April 2021 and 16 September 2021, and was consequently provided (see 'Documentation provided to EFSA').

### 2.2. Methodologies

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

The current Guidance on the submission of a dossier on food enzymes for safety evaluation (EFSA, 2009a) as well as the Statement on characterisation of microorganisms used for the production of food enzymes (EFSA CEP Panel, 2019) have been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance to the updated Scientific Guidance for the submission of dossiers on food enzymes (EFSA CEP Panel, 2021a).

## 3. Assessment

IUBMB nomenclature	Glucan 1,4- $\alpha$ -glucosidase
Systematic name	4- $\alpha$ -D-glucan glucohydrolase
Synonyms	glucoamylase; amyloglucosidase; exo-1,4- $\alpha$ -glucosidase
IUBMB No	EC 3.2.1.3
CAS No	9032-08-0
EINECS No	232-877-2

The glucan 1,4- $\alpha$ -glucosidase catalyses the hydrolysis of terminal (1-4)-linked  $\alpha$ -D-glucose residues successively from non-reducing ends of amylopectin and amylose with the release of glucose. The enzyme is intended to be used in starch processing for the production of glucose syrups and in distilled alcohol production.

### 3.1. Source of the food enzyme

The glucan 1,4- $\alpha$ -glucosidase is produced with the genetically modified filamentous fungus *Aspergillus niger* strain NZYM-BR, which is deposited at the German Collection of Microorganisms and Cell Cultures GmbH (DSMZ, Germany), with deposit number [REDACTED]

#### 3.1.1. Characteristics of the parental and recipient microorganism

[REDACTED] strains BO-1 [REDACTED], derived from the parental strain by classical mutagenesis, were found not to produce ochratoxin A and fumonisin B2 under conditions known to induce mycotoxin production in fungi.<sup>5</sup> [REDACTED]

<sup>4</sup> Technical dossier/Spontaneous data submission, November 2020/Annex v.2.

<sup>5</sup> Technical dossier/Annex A3.

<sup>6</sup>

[Redacted text block]

### 3.1.2. Characteristics of introduced sequences

[Redacted text block]

### 3.1.3. Description of the genetic modification process

[Redacted text block]

<sup>8</sup>

### 3.1.4. Safety aspects of the genetic modification

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

<sup>6</sup> Technical dossier/Annex A1.

<sup>7</sup> Technical dossier/Annex A2.

<sup>8</sup> Technical dossier/Annex D1.

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 852/2004<sup>9</sup>, with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.

The production strain is grown as a pure culture using a typical industrial medium in a submerged, fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration, leaving a supernatant containing the food enzyme. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in which the enzyme protein is retained, while most of the low molecular mass material passes the filtration membrane and is discarded.<sup>10</sup> The applicant provided information on the identity of the substances used to control the fermentation and in the subsequent downstream processing of the food enzyme.<sup>11</sup>

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- $\alpha$ -glucosidase is a single polypeptide chain of 556 amino acids.<sup>12</sup> The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be 59.1 kDa.<sup>12</sup> The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). A consistent protein pattern was observed across all batches. The gels showed a major protein band corresponding to a molecular mass of about 54 kDa, and few bands with lower staining intensity.<sup>13</sup> The food enzyme was tested for  $\alpha$ -amylase, lipase, pullulanase and protease activities and only  $\alpha$ -amylase activity was detected.<sup>14</sup>

The in-house determination of glucan 1,4- $\alpha$ -glucosidase activity is based on hydrolysis of maltose (reaction conditions: pH 4.3, temperature 37°C, reaction time 6 min). The enzymatic activity is quantified measuring the formation of glucose by means of an enzymatic assay. The glucan 1,4- $\alpha$ -glucosidase activity is quantified relative to an internal enzyme standard and expressed in Amyloglucosidase Units/g (AGU/g).<sup>15</sup>

The food enzyme has a temperature optimum around 65°C (pH 5.0) and a pH optimum around pH 4.0 (37°C). Its thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures (pH 5.0). The activity decreased above 55°C, showing no residual activity above 80°C.<sup>16</sup>

#### 3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation and one batch produced for the toxicological tests (Table 1).<sup>17</sup> The average total organic solids (TOS) of the three batches for commercialisation was 10.9% and the average enzyme activity/TOS ratio was 3.6 AGU/mg TOS.

<sup>9</sup> 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.

<sup>10</sup> Technical dossier/p. 49–55.

<sup>11</sup> Technical dossier/Annex 6 and Spontaneous data submission, November 2020/Annex v.2.

<sup>12</sup> Technical dossier/p. 33/Annex 1.

<sup>13</sup> Technical dossier/p. 35.

<sup>14</sup> Technical dossier/p. 45.

<sup>15</sup> Technical dossier/Annex 3.01.

<sup>16</sup> Technical dossier/p. 41–42/Annex 9.

<sup>17</sup> Technical dossier/p. 62/Spontaneous data submission, November 2020/Annex 7.03.

**Table 1:** Composition of the food enzyme

Parameters	Unit	Batches			
		1	2	3	4 <sup>(a)</sup>
<b>Glucan 1,4-<math>\alpha</math>-glucosidase activity</b>	AGU/g batch <sup>(b)</sup>	383	412	368	381
<b>Protein</b>	%	9.4	10.2	9.2	8.9
<b>Ash</b>	%	0.6	0.6	0.8	0.5
<b>Water</b>	%	87.9	87.4	89.9	88.6
<b>Total organic solids (TOS)<sup>(c)</sup></b>	%	11.5	12.0	9.3	10.9
<b>Activity/mg TOS</b>	AGU/mg TOS	3.3	3.4	4.0	3.5

(a): Batch used for the toxicological studies.

(b): AGU: glucan 1,4- $\alpha$ -glucosidase Units (see Section 3.3.1).

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

### 3.3.3. Purity

The lead content in the three commercial batches and in the batch used for toxicological studies was below 0.5 mg/kg, which complies with the specification for lead ( $\leq 5$  mg/kg) as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). In addition, the levels of cadmium and mercury were below the limits of detection (LoDs) of the employed methodologies.<sup>18,19</sup> For arsenic, the average concentration determined in the commercial batches was 0.19 mg/kg.<sup>20</sup> The Panel considered this concentration as not of concern.

The food enzyme complies with the microbiological criteria (for total coliforms, *Escherichia coli* and *Salmonella*) as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). No antimicrobial activity was detected in any of these batches.<sup>21</sup>

Strains of *Aspergillus*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Frisvad et al., 2018). The presence of the mycotoxins fumonisin B2 and ochratoxin A was examined in the three commercial food enzyme batches and both were below the LoD of the applied method.<sup>21,22</sup> The potential presence of other secondary metabolites is addressed by the toxicological examination of the food enzyme TOS.

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

[REDACTED]

<sup>23</sup>

The absence of recombinant DNA in the food enzyme concentrate was demonstrated

[REDACTED]

<sup>24</sup>

## 3.4. Toxicological data

A battery of toxicological tests including a bacterial gene mutation assay (Ames test), an *in vitro* micronucleus test and a repeated dose 90-day oral toxicity study in rats has been provided. The batch 4 (Table 1) used in these studies has a similar protein pattern and chemical purity as the commercial batches, and thus is considered suitable as a test item.

<sup>18</sup> LoDs: Pb = 0.5 mg/kg; Cd = 0.05 mg/kg; Hg = 0.03 mg/kg.

<sup>19</sup> Technical dossier/p. 62/Spontaneous data submission, November 2020.

<sup>20</sup> LoD: As = 0.1 mg/kg.

<sup>21</sup> Technical dossier/p. 63/Spontaneous data submission, November 2020.

<sup>22</sup> LoDs: fumonisin B2 = 0.0005 mg/kg, ochratoxin A = 0.0003 mg/kg.

<sup>23</sup> Technical dossier/Annex E1.

<sup>24</sup> Technical dossier/Additional Information, June 2021/Annex E2 - Revised.



### 3.4.1. Genotoxicity

#### 3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was performed according to the Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997) and following Good Laboratory Practice (GLP).<sup>25</sup> 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 (S9-mix), applying the 'treat and plate' assay. Two separate experiments were carried out using six concentrations of the food enzyme (from 156 to 5,000  $\mu\text{g}$  dry matter/plate, corresponding to 149, 299, 598, 1,195, 2,390 and 4,781  $\mu\text{g}$  TOS/plate). No cytotoxicity was observed at any concentration of the test substance. Upon treatment with the food enzyme, there was no biologically relevant increase in the number of revertant colonies above the control values in any strain tested, with or without S9-mix.

The Panel concluded that the food enzyme glucan 1,4  $\alpha$ -glucosidase did not induce gene mutations under the test conditions employed in this study.

#### 3.4.1.2. *In vitro* mammalian cell micronucleus test

The *in vitro* mammalian cell micronucleus test was carried out according to the OECD Test Guideline 487 (OECD, 2010) and following GLP.<sup>26</sup> A dose-range finding study was performed at concentrations up to 5,000  $\mu\text{g}/\text{mL}$  and no inhibition of cell growth by 50% or higher was observed. Based on these results, duplicate cultures of human peripheral blood lymphocytes were exposed to the food enzyme for 3 h in the presence or absence of the S9-mix and harvested 24 h after the beginning of treatment (3 + 21 h recovery time). Additionally, a continuous 24-h treatment without S9 mix was included with harvesting 24 h after removal of the test substance (24 + 24 h recovery time). The food enzyme was tested at 3,000, 4,000 and 5,000  $\mu\text{g}/\text{mL}$ , corresponding to 327, 436 and 545  $\mu\text{g}$  TOS/mL. No cytotoxicity was seen either in the short-term with or without S9-mix or in the long-term treatment. The frequency of binucleated cells with micronuclei (MNBN) was not statistically significantly different from the negative controls at all concentrations tested.

The Panel concluded that under the test conditions employed in this study the food enzyme glucan 1,4- $\alpha$ -glucosidase did not induce an increase in the frequency of MNBNs in cultured human peripheral blood lymphocytes.

### 3.4.2. Repeated dose 90-day oral toxicity study in rodents

The repeated dose 90-day oral toxicity study was performed in accordance with the OECD Test Guideline 408 (OECD, 1998) and following GLP.<sup>27</sup> Groups of 10 male and 10 female Sprague-Dawley (CrI:CD(SD)) rats received by gavage the food enzyme at doses of 113, 374 and 1,135 mg TOS/kg body weight (bw) per day. Controls received the vehicle (water for formulation).

One low-dose male was killed for welfare reasons after showing a swollen left hind paw (week 12). Histopathological examination revealed moderate inflammation in this paw. The Panel considered the reason for removing this animal from the experiment as not related to the test article.

The overall body weight gain of low-dose males was 9% lower than that of the controls. The Panel considered this change as not toxicologically relevant, as it was only observed in one sex, there was no dose-response relationship and the magnitude of the change was low and without a statistically significant effect on the final body weight.

In the functional observations, the high beam and low beam scores (rearing and cage floor activity, respectively) were statistically significantly lower in treated males than those of controls at some of the 6-minute intervals (6, 18 and 24 min), but the total scores for the 1-h recording period did not show any dose-response relationship and were not significantly different from controls. The Panel considered these intergroup differences as not toxicologically relevant, as they were transient and only observed in one sex.

Haematological examination revealed a statistically significant increase in haematocrit (+ 5%, + 5%) and haemoglobin (+ 5%, + 6%) concentrations in low- and mid-dose males, shortened prothrombin time (–5%) in low-dose females and a statistically significant increase in mean cell haemoglobin (MCH, + 3%, + 2%, + 2%) and mean cell volume (MCV, + 3%, + 3%, + 2%) in low-, mid- and high-dose females. The Panel considered these changes as not toxicologically relevant because they were observed only in one

<sup>25</sup> Technical dossier/Annex 7.01.

<sup>26</sup> Technical dossier/Annex 7.02.

<sup>27</sup> Technical dossier/Annex 7.03.

sex (all parameters), were of low magnitude (all parameters) and lacked an apparent dose–response relationship (all parameters).

Clinical chemistry investigation revealed a statistically significant increase in aspartate amino-transferase activity (+ 21%) in high-dose males. The Panel considered this change as not toxicologically relevant because of the low magnitude of the change, its observation only in one sex and being within the historical control values.

Statistically significant changes in organ weights were limited to a decrease in adjusted spleen weight in low-, mid- and high-dose males (–14%, –13% and –20%). The Panel noted that also the absolute spleen weights were decreased in low-, mid- and high-dose males (–18%, –14%, –20%), although the changes did not reach statistical significance and were without an apparent dose–response relationship. The Panel considered these changes as not toxicologically relevant due to the absence of a dose–response relationship, the observation in only one sex, the absence of histopathological changes in the spleen and the absence of corroborative changes in clinical pathology or in the weight and histopathology of the thymus.<sup>28</sup>

The microscopic examination revealed a slight exacerbation of multifocal myocardial inflammation/ degeneration in high-dose males (4/10 vs. 0/10). The Panel considered these changes not to be toxicologically relevant because they were of minimal severity, occurred only in one sex and were within the laboratory historical control data.

An increased incidence of hyaline droplets in the kidneys of mid- and high-dose males (8/10 and 10/10 vs. 5/10) was observed. The Panel considered these changes not to be toxicologically relevant because it is a common male rat specific finding not relevant for human risk assessment.

No other statistically significant or biologically relevant differences to controls were reported.

The Panel identified the no observed adverse effect level (NOAEL) of 1,135 mg TOS/kg bw per day, the highest dose tested.

### 3.4.3. Allergenicity

The allergenicity assessment considers only the food enzyme and not carriers or other excipients, which may be used in the final formulation.

The potential allergenicity of the glucan 1,4- $\alpha$ -glucosidase produced with the genetically modified *A. niger* strain NZYM-BR was assessed by comparing its amino acid sequence with those of known allergens according to the Scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms (EFSA GMO Panel, 2010). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, two matches were found.<sup>29</sup> The matching allergens were Asp o 21, an  $\alpha$ -amylase produced by *A. oryzae* and Sch c 1, a glucoamylase (glucan 1,4- $\alpha$ -glucosidase) produced by *Schizophyllum commune*.

Both glucan 1,4- $\alpha$ -glucosidase from *S. commune* (Toyotome et al., 2014) and  $\alpha$ -amylase from *A. oryzae* (Brisman and Belin, 1991; Sander et al., 1998; Quirce et al., 2002; Brisman, 2002) are known as occupational respiratory allergens associated with baker's asthma. However, several studies have shown that adults with occupational asthma caused by an enzyme (as described for  $\alpha$ -amylase from *A. oryzae*) can ingest respiratory allergens without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Poulsen, 2004; Armentia et al., 2009). Considering the wide use of  $\alpha$ -amylase as a food enzyme, only a low number of cases have been reported in the literature on allergic reactions upon oral exposure in individuals respiratory-sensitised to  $\alpha$ -amylase (Losada et al., 1992; Quirce et al., 1992; Baur and Czuppon, 1995; Kanny and Moneret-Vautrin, 1995; Moreno-Ancillo et al., 2004). Such information has not been reported for glucan 1,4- $\alpha$ -glucosidase.

No information is available on oral and respiratory sensitisation or elicitation reactions of the glucan 1,4- $\alpha$ -glucosidase under evaluation.

According to the information provided, substances or products that may cause allergies or intolerances (Regulation (EU) No 1169/2011<sup>30</sup>) are used as raw materials (██████████). In addition,

<sup>28</sup> Technical dossier/Additional information, June 2021/90-day study LKG0032 Additional data.

<sup>29</sup> Technical dossier/Additional information, June 2021/Annex 8-01 and 8-02.

<sup>30</sup> 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.

known allergens, are present in the media fed to the microorganisms. However, during the fermentation process, these products are degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the fungal biomass and fermentation solids are removed. Taking into account the fermentation process and the downstream processing, the Panel considered that potentially allergenic residues of these proteins are not expected to be present.

The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme can be excluded for distilled alcohol production. The risk cannot be excluded for starch processing for glucose syrup production, but the likelihood of such reactions to occur is considered to be low.

### 3.5. Dietary exposure

#### 3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in two food processes at the recommended use levels summarised in Table 2.<sup>4</sup>

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

Food manufacturing process <sup>(a)</sup>	Raw material	Recommended dosage of the food enzyme
Starch processing for glucose syrup production	Starch	up to 98 mg TOS/kg starch
Distilled alcohol production	Cereals	up to 202 mg TOS/kg cereals

TOS: total organic solids.

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

In starch processing, the food enzyme is added during the saccharification step, where it degrades gelatinised starch into dextrins.<sup>31</sup> The food enzyme can also be used to further degrade the dextrins to fermentable sugars. The food enzyme-TOS is removed from the final glucose syrups by treatment with activated charcoal or similar and with ion-exchange resins (EFSA CEP Panel, 2021b).

In distilled alcohol production, the food enzyme is applied together with other saccharification enzymes to degrade the dextrins to fermentable sugars.<sup>32</sup> In plants using the simultaneous saccharification and fermentation process, liquefied mash is pumped into the fermenter, where the food enzyme and other saccharifying enzymes are added together with yeast at the start of the fermentation. The food enzyme-TOS is not carried over with the distilled alcohols (EFSA CEP Panel, 2021b).

#### 3.5.2. Dietary exposure estimation

The Panel accepted the evidence provided as sufficient to conclude that the residual amounts of food enzyme-TOS in the final distilled alcohol and glucose syrups is negligible. Consequently, a dietary exposure was not calculated.

### 3.6. Margin of exposure

In the absence of an estimate for the dietary exposure, the margin of exposure is not calculated.

## 4. Conclusions

Based on the data provided and the removal of TOS during the intended food production processes, the Panel concluded that the food enzyme glucan 1,4- $\alpha$ -glucosidase produced with the genetically modified *A. niger* strain NZYM-BR does not give rise to safety concerns under the intended conditions of use.

<sup>31</sup> Technical dossier/p. 80.

<sup>32</sup> Technical dossier/p. 82.

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

## 5. Documentation as provided to EFSA

- 1) Technical dossier "Amyloglucosidase from a genetically modified strain of *Aspergillus niger* (strain NZYM-BR)". 24 June 2013. Submitted by Novozymes A/S.
- 2) Additional information. 25 June 2021. Submitted by Novozymes A/S.
- 3) Additional information. 15 December 2021. Submitted by Novozymes A/S.
- 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 (AMFEP). Unpublished document.

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## Abbreviations

AGU	Amyloglucosidase Unit
5-ALA	5-aminolevulinic acid
bp	base pair
bw	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
DSMZ	Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturep GmbH
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
FOA	5-fluoro-orotic-acid
GLP	Good Laboratory Practice
GM	genetically modified
GMO	genetically modified organism
ITS	internal transcribed spacer
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LoD	limit of detection
MCH	mean cell haemoglobin
MCV	mean cell volume
MNBN	binucleated cells with micronuclei
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
S9-mix	metabolic activation
TOS	total organic solids
WHO	World Health Organization