Safety evaluation of the food enzyme glucan 1,4-α-glucosidase from Aspergillus niger

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Abstract

The food enzyme glucan 1,4-α-glucosidase (4-α-D-glucan glucohydrolase, EC 3.2.1.3) is produced with a non-genetically modified strain of Aspergillus niger by Beijing Shifa Technology & Trade Co., Ltd. The food enzyme is intended to be used in baking processes, cereal-based processes, brewing processes, fruit and vegetable processing for juice production, distilled alcohol production, starch processing for the production of glucose syrups and other starch hydrolysates, and yeast processing. In the absence of data sufficient to characterise the source of food enzyme, its method of production, its chemical characterization and its potential allergenicity, coupled with the absence of study reports for toxicological data and insufficient information about food manufacturing processes, the Panel was unable to assess the safety of the food enzyme glucan 1,4-α-glucosidase from an unknown strain of Aspergillus niger.

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Keywords: glucan 1,4-α-glucosidase, 4-α-D-glucan glucohydrolase, EC 3.2.1.3, Aspergillus niger, non-genetically modified microorganism

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1. Introduction

Article 3 of the Regulation (EC) No 1332/2008 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 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 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 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.

Five applications have been introduced by the company ‘Amano Enzyme Inc.’ and the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) for the authorisation of the food enzymes ribonuclease P from *Penicillium citrinum* (strain AE-RP), glutaminase from *Bacillus amyloliquefaciens* (strain AE-GT), oryzin from *Aspergillus melleus* (strain AE-P), triacylglycerol lipase from *Candida rugosa* (strain AE-LAY) and glucoamylase from *Aspergillus niger*, respectively.

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011 implementing Regulation (EC) No 1331/2008, the Commission has verified that the five applications fall within the scope of the food enzyme Regulation and contain all the elements required under Chapter II of that Regulation.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes ribonuclease P from *Penicillium citrinum* (strain AE-RP), glutaminase from *Bacillus amyloliquefaciens* (strain AE-GT), oryzin from *Aspergillus melleus* (strain AE-P),

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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, p. 15–24.
triacylglycerol lipase from *Candida rugosa* (strain AE-LAY) and glucoamylase from *Aspergillus niger* in accordance with Article 17.3 of Regulation (EC) No 1332/2008\(^4\) 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 glucan 1,4-α-glucosidase (glucoamylase) from *A. niger*.

The application was submitted initially as a joint dossier\(^4\) and identified as the EFSA-Q-2015-00292. During the risk assessment phase, it was found that the technical dossier is too generic to be evaluated. A solution was found on 16 March 2020 via an ad hoc meeting between EFSA, the European Commission and representatives from the Association of Manufacturers and Formulators of Enzyme Products (AMFEP).\(^5\) It was agreed that a joint dossier will be split into individual data packages.

The current opinion addresses one data package originating from the joint dossier EFSA-Q-2015-00292. This data package, identified as EFSA-Q-2020-00795, concerns the food enzyme glucan 1,4-α-glucosidase that is produced with a strain of *A. niger* and submitted by Beijing Shifa Technology & Trade Co., Ltd.

2. Data and methodologies

2.1. Data

The applicant submitted an independent data package in support of the application for authorisation of the food enzyme glucan 1,4-α-glucosidase from *A. niger* on 8 December 2020.

Data submitted in the application EFSA-Q-2020-00795 differs little from the original joint dossier. In order to progress with the risk assessment, EFSA invited the applicant to a clarification teleconference on 2 December 2021 and 8 December 2021. The teleconference would have aimed to clarify the information provided in the data package, in particular regarding: quality of the dossier provided in general, lack of information as requested by the applicable guidance, study reports, certificates of analysis and all supportive documents indicated as Annexes in the dossier, which are missing. However, EFSA has never received a reply from the applicant.

A final reminder was sent by EFSA on 12 January 2022, again no reply was received. Therefore, under these circumstances, on 22 February 2022, EFSA communicated to the applicant that EFSA is going to finalise the evaluation of the application EFSA-Q-2020-00795 based on the information provided in the data package on 8 December 2020.

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 guidance documents of EFSA Scientific Committee.

The current ‘Guidance on the submission of a dossier on food enzymes for safety evaluation’ (EFSA CEF Panel, 2009) 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, 2021).

\(^4\) A Joint Dossier is permitted by the Commission Implementing Regulation (EU) No 562/2012 of 27 June 2012 amending Commission Regulation (EU) No 234/2011 with regard to specific data required for risk assessment of food enzymes. OJ L 168, 28.6.2012, p. 21–23.

\(^5\) The full details are available online: https://www.efsa.europa.eu/en/events/event/ad-hoc-meeting-industry-association-amfep-joint-dossiers-food-enzymes
3. Assessment

| IUBMB nomenclature | Glucan 1,4-α-glucosidase |
|---------------------|--------------------------|
| Systematic name     | 4-α-D-glucan glucohydrolase |
| Synonyms            | glucoamylase, amyloglucosidase |
| IUBMB No            | EC 3.2.1.3 |
| CAS No              | 9032-08-0 |
| EINECS No           | 232-877-2 |

Glucan 1,4-α-glucosidase catalyses the hydrolysis of (1->4)-linked α-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 baking processes, cereal-based processes, brewing processes, fruit and vegetable processing for juice production, distilled alcohol production, starch processing for glucose syrups production and other starch hydrolysates, and yeast processing.7

3.1. Source of the food enzyme

The glucan 1,4-α-glucosidase is produced with the non-genetically modified filamentous fungus A. niger.

The applicant did not provide the name, code or unique identifier of the specific strain used for the production of the food enzyme, any certificate of deposition or any characterisation of the production strain. This fails to meet the basic requirements of the EFSA ‘Statement on characterisation of microorganisms used for the production of food enzymes’ (EFSA CEP Panel, 2019).

3.2. Production of the food enzyme

The applicant states that food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004,10 with food safety procedures based on hazard analysis and critical control points,11 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, batch or fed-batch fermentation system12 or in a solid-state system, with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration in the case of the liquid system and after the addition of water in the case of the solid-state fermentation, both leaving a supernatant containing the food enzyme. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step13 in which enzyme protein is retained, while most of the low molecular mass material passes the filtration membrane and is discarded.

The applicant did not provide information on the identity of the substances used to control the fermentation and on the subsequent downstream processing of the food enzyme.

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

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6 Technical dossier/p. 4-8, 27, 60.
7 Technical dossier/p. 4, 9, 15.
8 Technical dossier/p. 7, 33-37.
9 Technical dossier/p. 7-8, 14, 37-44.
10 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.
11 Technical dossier/p. 36.
12 Technical dossier/p. 38.
13 Technical dossier/p. 41.
3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

According to published studies, glucan 1,4-\(\alpha\)-glucosidases have a single polypeptide chain of 639–640 amino acids.\(^{15}\) The molecular mass of the mature protein, calculated from the published amino acid sequence, is 68.3 kDa.\(^{15}\) Three batches of the food enzyme were analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis.\(^{16}\) A consistent protein pattern was observed across all batches.\(^{16}\) The gels showed two bands, one corresponding to the native protein, and one assumed to correspond to the glycosylated form of the enzyme protein, which migrated above the 97 kDa marker.\(^{17}\) No other enzymatic activities were reported by the applicant.\(^{18}\)

The in-house method for the determination of the enzyme activity and the definition of units of activity were not provided by the applicant.

The food enzyme has a temperature optimum around 55–65°C (pH 5) and a pH optimum around pH 3–5 (37°C).\(^{19}\) Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures (pH 5). Glucan 1,4-\(\alpha\)-glucosidase activity was stable up to 55°C, but enzyme activity was lost after incubation at 70°C or above.\(^{20}\)

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (Table 1). The mean total organic solids (TOS) of the three food enzyme batches for commercialisation is 23.1% and the mean enzyme activity/mg TOS ratio is 1.6 AGU/mg TOS.

Table 1: Composition of the food enzyme

| Parameters                                    | Unit               | Batch 1 | Batch 2 | Batch 3 |
|-----------------------------------------------|--------------------|---------|---------|---------|
| Glucan 1,4-\(\alpha\)-glucosidase activity    | AGU/g batch\(^{(a)}\) | 336     | 432     | 316     |
| Protein                                       | %                  | 10.3    | 14.6    | 9.7     |
| Ash                                           | %                  | 0.6     | 0.6     | 0.6     |
| Water                                         | %                  | 78      | 72      | 79      |
| Total organic solids (TOS)\(^{(b)}\)          | %                  | 21.4    | 27.4    | 20.4    |
| Glucan 1,4-\(\alpha\)-glucosidase activity/mg TOS | AGU/mg TOS         | 1.57    | 1.58    | 1.55    |

\(^{(a)}\): AGU: AmyloGlucosidase Units (Technical dossier/p. 27–28).
\(^{(b)}\): TOS calculated as 100% – % water – % ash.

The applicant did not provide the certificates of analysis for the chemical parameters shown in Table 1.

3.3.3. Purity

The lead content\(^{23}\) in the three commercial batches was below 5 mg/kg which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).

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\(^{14}\) Technical dossier/p. 7, 30.
\(^{15}\) Technical dossier/p. 30; ExPASY, Brenda and UniProtKB.
\(^{16}\) Technical dossier/p. 28, 30.
\(^{17}\) Technical dossier/p. 30.
\(^{18}\) Technical dossier/p. 6, 31.
\(^{19}\) Technical dossier/p. 7, 31–32.
\(^{20}\) Technical dossier/p. 31–32.
\(^{21}\) Technical dossier/p. 27–28, 57–58.
\(^{22}\) Technical dossier/p. 6–7, 29–30, 57–58, 61.
\(^{23}\) Technical dossier/p. 6, 30, 57–58, 61.
The food enzyme complies with the microbiological criteria (for total coliforms, *Escherichia coli* and *Salmonella*)\(^{24}\) as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). No antimicrobial activity was detected in any of the tested batches.\(^{25}\)

The applicant did not provide the analytical method or the certificates of analysis for any parameter regarding food enzyme purity. The Panel considered that the information provided on the purity of the food enzyme is not sufficient.

### 3.3.4. Viable cells of the production strain

No data on the absence of viable cells of the production strain in the food enzyme was provided.

### 3.4. Toxicological data\(^ {26}\)

The results of toxicological studies made with the food enzyme were reported only in summary form. In the absence of the full study reports, the Panel was unable to proceed with the evaluation of toxicity.

#### 3.4.1. Allergenicity\(^ {27}\)

No assessment of allergenicity was provided by the applicant.

### 3.5. Dietary exposure

#### 3.5.1. Intended use of the food enzyme\(^ {28}\)

The food enzyme is intended to be used in seven food processes at the recommended use levels summarized in Table 2.\(^ {29}\)

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

| Food manufacturing process\(^ {(a)}\) | Raw material (RM) | Range of the recommended use level (mg TOS/kg RM) |
|-------------------------------------|------------------|-----------------------------------------------|
| Baking processes                    | Flour            | 6–400                                         |
| Cereal-based processes              | Flour            | 6–400                                         |
| Brewing processes                   | Cereals          | 33–300                                        |
| Fruit and vegetable processing for juice production | Fruits/vegetables | 8–35                                          |
| Distilled alcohol production        | Cereals          | 33–300                                        |
| Starch processing for the production of starch hydrolysates | Cereals | 2–248                                         |
| Yeast processing (autolysed) Yeast  | (autolysed) Yeast| 10–1,000                                      |

TOS: total organic solids.

\(^{(a)}\): The description has been harmonized by EFSA according to the 'EC working document describing the food processes in which food enzymes are intended to be used’ – not yet published at the time of adoption of this opinion.

Although EFSA was able to harmonise the description of the intended food manufacturing processes based on information provided by the applicant, information is missing for several details, such as the confirmation of the use of ‘cereal-based processes’ and clarification on the raw material for the ‘starch processing’. The technical dossier also does not contain sufficient technical information about the use of the glucan 1,4-α-glucosidase in yeast processing.

\(^{24}\) Technical dossier/p. 7, 30, 57, 61.

\(^{25}\) Technical dossier/p. 7, 57, 61.

\(^{26}\) Technical dossier/p. 10, 14, 55–58.

\(^{27}\) Technical dossier/p. 10–11, 58–59.

\(^{28}\) Technical dossier/p. 4, 8–9, 11–13, 15, 45–46, 51–52, 73–74.

\(^{29}\) Technical dossier/p. 46–52.
### 3.5.2. Dietary exposure estimation

The lack of clarity in Table 2 and the limited technical information about the use of the food enzyme in yeast processing preclude an estimate of the dietary exposure.

### 4. Conclusions

In the absence of data sufficient to characterise the source of food enzyme, its method of production, its chemical characterization and its potential allergenicity, coupled with the absence of study reports for toxicological data and insuffi cient information about food manufacturing processes, the Panel was unable to assess the safety of the food enzyme glucan 1,4-α-glucosidase from an unknown strain of *Aspergillus niger*.

### 5. Documentation as provided to EFSA

1) Technical dossier ‘Application for authorization of glucoamylase from *Aspergillus niger* in accordance with Regulation (EC) 1331/2008’ (an independent data package of the original joint dossier 2015-00292). 25 November 2020. Submitted by Beijing Shifa Technology & Trade Co., Ltd.

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### Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| AGU          | AmyloGlucosidase Unit |
| AMFEP        | Association of Manufacturers and Formulators of Enzyme Products |
| CAS          | Chemical Abstracts Service |
| CEF          | EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids |
| CEP          | EFSA Panel on Food Contact Materials, Enzymes and Processing Aids |
| EINECS       | European Inventory of Existing Commercial Chemical Substances |
| FAO          | Food and Agricultural Organization of the United Nations |
| IUBMB        | International Union of Biochemistry and Molecular Biology |
| JECFA        | Joint FAO/WHO Expert Committee on Food Additives |
| RM           | raw material |
| SDS-PAGE     | sodium dodecyl sulfate-polyacrylamide gel electrophoresis |
| TOS          | total organic solids |
| WHO          | World Health Organization |