

ADOPTED: 14 September 2022

doi: 10.2903/j.efsa.2022.7571

## Safety evaluation of the food enzyme $\beta$ -galactosidase from the non-genetically modified *Kluyveromyces lactis* strain AE-KL

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP),  
Claude Lambré, José Manuel Barat Baviera, Claudia Bolognesi, Pier Sandro Cocconcelli,  
Riccardo Crebelli, David Michael Gott, Konrad Grob, Evgenia Lampi, Marcel Mengelers,  
Alicja Mortensen, Gilles Rivière, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren,  
Laurence Vernis, Holger Zorn, Boet Glandorf, Lieve Herman, Natália Kovalkovičová, Yi Liu,  
Giulio di Piazza, Rita Ferreira de Sousa and Andrew Chesson

### Abstract

The food enzyme  $\beta$ -galactosidase ( $\beta$ -D-galactoside galactohydrolase, EC 3.2.1.23) is produced with the non-genetically modified *Kluyveromyces lactis* strain AE-KL by Amano Enzyme Inc. As the production strain meets the requirements for a Qualified Presumption of Safety (QPS) approach to safety assessment and as no other issues of concern were identified, the Panel considered that toxicological tests were not needed for the assessment of this food enzyme. The food enzyme is intended to be used for lactose hydrolysis in milk processing (including infant formulae), production of fermented milk products and manufacture of galacto-oligosaccharides (GOS). The dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 7.933 mg TOS/kg body weight (bw) per day in European populations. A search for similarity of the amino acid sequence of the food enzyme to known allergens was made and no match was found. The Panel considered that, under the intended conditions of use, the risk of allergic reactions by dietary exposure cannot be excluded, but the likelihood for this to occur is considered to be low. Based on the QPS status of the production strain and the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns, under the intended conditions of use.

© 2022 European Food Safety Authority. *EFSA Journal* published by Wiley-VCH GmbH on behalf of European Food Safety Authority.

**Keywords:** food enzyme,  $\beta$ -galactosidase,  $\beta$ -D-galactoside galactohydrolase, EC 3.2.1.23, *Kluyveromyces lactis*, non-genetically modified microorganism

**Requestor:** European Commission

**Question number:** EFSA-Q-2014-00669

**Correspondence:** [fip@efsa.europa.eu](mailto:fip@efsa.europa.eu)

**Panel members:** José Manuel Barat Baviera, Claudia Bolognesi, Andrew Chesson, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Claude Lambré, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Vittorio Silano (until 21 December 2020 †), Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis and Holger Zorn.

**Note:** The full opinion will be published in accordance with Article 12 of Regulation (EC) No 1331/2008 once the decision on confidentiality will be received from the European Commission.

**Declarations of interest:** If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact [interestmanagement@efsa.europa.eu](mailto:interestmanagement@efsa.europa.eu).

**Acknowledgements:** The Panel wishes to thank the following for the support provided to this scientific output: Jaime Aguilera, Magdalena Andryszkiewicz, Ana Gomes, Sandra Rainieri.

**Suggested citation:** EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré C, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mengelers M, Mortensen A, Rivière G, Steffensen I-L, Tlustos C, Van Loveren H, Vernis L, Zorn H, Glandorf B, Herman L, Kovalkovičová N, Liu Y, di Piazza G, Ferreira de Sousa R and Chesson A, 2022. Safety evaluation of the food enzyme  $\beta$ -galactosidase from the non-genetically modified *Kluyveromyces lactis* strain AE-KL. EFSA Journal 2022;20(10):7571, 14 pp. <https://doi.org/10.2903/j.efsa.2022.7571>

**ISSN:** 1831-4732

© 2022 European Food Safety Authority. EFSA Journal published by Wiley-VCH GmbH on behalf of European Food Safety Authority.

This is an open access article under the terms of the [Creative Commons Attribution-NoDerivs](https://creativecommons.org/licenses/by/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited and no modifications or adaptations are made.



The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.



---

† Deceased.

## Table of contents

|                                                                                |    |
|--------------------------------------------------------------------------------|----|
| Abstract.....                                                                  | 1  |
| 1. Introduction.....                                                           | 4  |
| 1.1. Background and Terms of Reference as provided by the requestor.....       | 4  |
| 1.1.1. Background as provided by the European Commission.....                  | 4  |
| 1.1.2. Terms of Reference.....                                                 | 5  |
| 1.2. Interpretation of the Terms of Reference.....                             | 5  |
| 2. Data and methodologies.....                                                 | 5  |
| 2.1. Data.....                                                                 | 5  |
| 2.2. Methodologies.....                                                        | 5  |
| 3. Assessment.....                                                             | 5  |
| 3.1. Source of the food enzyme.....                                            | 5  |
| 3.2. Production of the food enzyme.....                                        | 6  |
| 3.3. Characteristics of the food enzyme.....                                   | 6  |
| 3.3.1. Properties of the food enzyme.....                                      | 6  |
| 3.3.2. Chemical parameters.....                                                | 6  |
| 3.3.3. Purity.....                                                             | 7  |
| 3.4. Toxicological data.....                                                   | 7  |
| 3.4.1. Allergenicity.....                                                      | 7  |
| 3.5. Dietary exposure.....                                                     | 8  |
| 3.5.1. Intended use of the food enzyme.....                                    | 8  |
| 3.5.2. Dietary exposure estimation.....                                        | 9  |
| 3.5.3. Uncertainty analysis.....                                               | 9  |
| 3.6. Margin of exposure.....                                                   | 10 |
| 4. Conclusions.....                                                            | 10 |
| 5. Documentation as provided to EFSA.....                                      | 10 |
| References.....                                                                | 10 |
| Abbreviations.....                                                             | 12 |
| Appendix A – Dietary exposure estimates to the food enzyme–TOS in details..... | 13 |
| Appendix B – Population groups considered for the exposure assessment.....     | 14 |

## 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<sup>1</sup> 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 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<sup>1</sup> on food enzymes.

Five applications have been introduced by the companies 'Genencor International B.V.', 'Amano Enzyme Inc.' and 'DSM Food Specialties B.V.' for the authorisation of the food enzymes endo-1,4-beta-xylanase from *Aspergillus niger* expressed in a genetically modified strain of *Trichoderma reesei* (DP-Nzd22), acylglycerol lipase from *Penicillium camemberti* (strain AE-LG), beta-galactosidase from *Kluyveromyces lactis* (strain AE-KL), beta-galactosidase from *Bacillus circulans* (strain AE-LT) and arabinofuranosidase from *Aspergillus niger* (strain ARF).

Following the requirements of Article 12.1 of Commission Regulation (EU) No 234/2011<sup>3</sup> implementing Regulation (EC) No 1331/2008<sup>2</sup>, 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.

<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.

### 1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments of the food enzymes endo-1,4-beta-xylanase from *Aspergillus niger* expressed in a genetically modified strain of *Trichoderma reesei* (DP-Nzd22), acylglycerol lipase from *Penicillium camemberti* (strain AE-LG), beta-galactosidase from *Kluyveromyces lactis* (strain AE-KL), beta-galactosidase from *Bacillus circulans* (strain AE-LT) and arabinofuranosidase from *Aspergillus niger* (strain ARF) in accordance with Article 17.3 of Regulation (EC) No 1332/2008<sup>1</sup> 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 food enzyme  $\beta$ -galactosidase from the non-genetically modified *K. lactis* strain AE-KL.

## 2. Data and methodologies

### 2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme  $\beta$ -galactosidase from a non-genetically modified *K. lactis* strain AE-KL.

Additional information was requested from the applicant during the assessment process on 08 October 2020 and received on 31 March 2021 (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, 2009) and following the relevant guidance documents of the EFSA Scientific Committee.

The current 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA CEP 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 with the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021a).

## 3. Assessment<sup>4</sup>

|                     |                                                       |
|---------------------|-------------------------------------------------------|
| IUBMB nomenclature: | $\beta$ -galactosidase                                |
| Systematic name:    | $\beta$ -D-galactoside galactohydrolase               |
| Synonyms:           | lactase; $\beta$ -lactosidase; $\beta$ -D-lactosidase |
| IUBMB No.:          | 3.2.1.23                                              |
| CAS No.:            | 9031-11-2                                             |
| EINECS No.:         | 232-864-1                                             |

$\beta$ -Galactosidases catalyse the hydrolysis of the  $\beta$ -(1,4)-glycosidic linkage of, e.g. lactose ( $\beta$ -D-galactosyl-1,4-D-glucoside) and the transgalactosylation of lactose to generate galacto-oligosaccharides (GOS).

The food enzyme under evaluation is intended to be used in three food manufacturing processes, lactose hydrolysis in milk processing (including infant formulae), production of fermented milk products and the manufacture of GOS.

### 3.1. Source of the food enzyme<sup>5</sup>

The food enzyme  $\beta$ -galactosidase is produced with the non-genetically modified yeast *K. lactis* strain AE-KL, which is deposited in [REDACTED]

[REDACTED] with deposit number [REDACTED]<sup>6</sup>

The production strain was identified as *K. lactis* by [REDACTED]<sup>7</sup>

<sup>4</sup> Technical dossier/1st submission/p. 4–5, 9, 22, 26, 53–54; Technical dossier/Additional data, 31 March 2021.

<sup>5</sup> Technical dossier/1st submission/p. 5, 9, 29–30, 53; Technical dossier/Additional data, 31 March 2021/Annex 2.

<sup>6</sup> Technical dossier/Additional data, 31 March 2021/Annex 1.

<sup>7</sup> Technical dossier/Additional data, 31 March 2021/Annex 2.

The species *K. lactis* is included in the list of microorganisms considered suitable for the 'Qualified Presumption of Safety' approach to safety assessment (EFSA BIOHAZ Panel, 2020). As its identity has been established and no other qualifications are applied, the production strain meets the criteria for the QPS approach.

### 3.2. Production of the food enzyme<sup>8</sup>

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.<sup>10</sup>

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 and release of the intracellular enzyme, 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 most of the 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 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<sup>11</sup>

The food enzyme  $\beta$ -galactosidase is a single polypeptide chain of [REDACTED] amino acids. The molecular mass of the protein was calculated to be [REDACTED] kDa.<sup>12</sup> The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis. A consistent protein pattern was observed across all batches. The gels showed the target protein migrating between the marker proteins of [REDACTED] kDa in all batches consistent with the calculated mass of the enzyme.<sup>13</sup> No other enzymatic activities were reported by the applicant.<sup>14</sup>

The in-house determination of  $\beta$ -galactosidase activity<sup>15</sup> is based on hydrolysis of *o*-nitrophenol  $\beta$ -D-galactopyranoside (reaction conditions: pH 6.5, 30°C, 10 min). The enzymatic activity is determined by measuring the release of *o*-nitrophenol spectrophotometrically at 420 nm. The enzyme activity is expressed in U/g. One activity unit is defined as the quantity of enzyme required to liberate 1  $\mu$ mol of *o*-nitrophenol per one minute under the conditions of the assay.

The food enzyme has a temperature optimum around 40°C (pH 6.5) and a pH optimum around pH 6.5 (40°C). Thermostability was tested by pre-incubation of the food enzyme for 10 min at different temperatures (pH 6.5). Enzyme activity decreased above 40°C, showing no residual activity above 55°C.<sup>16</sup>

#### 3.3.2. Chemical parameters<sup>17</sup>

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 39.7% and the mean enzyme activity/mg TOS ratio is 53.6 U/mg TOS.

<sup>8</sup> Technical dossier/1st submission/p. 9–10, 16, 32–39; Technical dossier/1st submission/Annex 4, Annex 5, Annex 6; Technical dossier/Additional data, 31 March 2021.

<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, pp. 3–21.

<sup>10</sup> Technical dossier/1st submission/p. 9, 32, 38; Technical dossier/1st submission/Annex 4.

<sup>11</sup> Technical dossier/1st submission/p. 25, 27.

<sup>12</sup> Technical dossier/1st submission/p. 25.

<sup>13</sup> Technical dossier/1st submission/Annex 8.

<sup>14</sup> Technical dossier/1st submission/p. 24, 26.

<sup>15</sup> Technical dossier/1st submission/Annex 2.

<sup>16</sup> Technical dossier/1st submission/p. 9; 27; Technical dossier/1st submission/Annex 2.

<sup>17</sup> Technical dossier/1st submission/p. 22–23, Technical dossier/1st submission/Annex 1, Annex 2, Annex 3; Technical dossier/2nd submission, 30 January 2015; Technical dossier/Additional data, 31 March 2021/Annex 5.

**Table 1:** Compositional data of the food enzyme

| Parameters                                       | Unit                     | Batches |        |        |
|--------------------------------------------------|--------------------------|---------|--------|--------|
|                                                  |                          | 1       | 2      | 3      |
| <b><math>\beta</math>-Galactosidase activity</b> | U/g batch <sup>(a)</sup> | 17,400  | 20,900 | 23,900 |
| <b>Protein</b>                                   | %                        | 4.7     | 5.9    | 5.7    |
| <b>Ash</b>                                       | %                        | 0.1     | 0.2    | 0.3    |
| <b>Water</b>                                     | %                        | 53.7    | 66.4   | 60.1   |
| <b>Total organic solids (TOS)<sup>(b)</sup></b>  | %                        | 46.2    | 33.4   | 39.6   |
| <b>Activity/mg TOS</b>                           | U/mg TOS                 | 37.7    | 62.6   | 60.4   |

(a): U: UNIT (see Section 3.3.1).

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

### 3.3.3. Purity<sup>18</sup>

The lead content in the three commercial batches was below 0.005 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, arsenic, cadmium and mercury contents were below 0.049, 0.010 and 0.001 mg/kg, respectively.<sup>19</sup> The Panel considered these concentrations 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).<sup>20,21</sup> No antimicrobial activity was detected in any of the three tested batches.<sup>22</sup>

The presence of aflatoxins B1, B2, G1 and G2, deoxynivalenol, HT-2 toxin, T-2 toxin, zearalenone, ochratoxin A and sterigmatocystin was examined in three food enzyme batches. All were below the limits of quantification (LOQs) of the applied analytical methods.<sup>23</sup>

The Panel considered that the information provided on the purity of the food enzyme is sufficient.

## 3.4. Toxicological data<sup>24</sup>

As the production strain qualifies for the QPS approach of safety assessment and as no issues of concern arising from the production process of the food enzyme were identified (see Sections 3.1, 3.2 and 3.3), the Panel considered that no toxicological studies other than the assessment of allergenicity were necessary (EFSA CEP Panel, 2021a).

### 3.4.1. Allergenicity<sup>25</sup>

The allergenicity assessment considered only the food enzyme and not any carrier or other excipients which may be used in the final formulation.

The potential allergenicity of the food enzyme  $\beta$ -galactosidase produced with the non-genetically modified *K. lactis* strain AE-KL 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, no match was found.<sup>26</sup>

<sup>18</sup> Technical dossier/1st submission/p. 8–9, 25, 53, Technical dossier/1st submission/Annex 1, Annex 3; Technical dossier/2nd submission, 30 January 2015.

<sup>19</sup> Technical dossier/1st submission/p. 24, 53; Technical dossier/1st submission/Annex 1, Annex 3; Technical dossier/2nd submission, 30 January 2015/Annex 1: LOQ: Pb = 0.005 mg/kg; As = 0.002 mg/kg; Cd = 0.001 mg/kg; Hg = 0.001 mg/kg.

<sup>20</sup> Technical dossier/1st submission/p. 8–9, 25, 53; Technical dossier/1st submission/Annex 1, Annex 3.

<sup>21</sup> Technical dossier/1st submission/Annex 3.

<sup>22</sup> Technical dossier/1st submission/p. 25, 53; Technical dossier/1st submission/Annex 1, Annex 3; Technical dossier/2nd submission, 30 January 2015/Annex 1.

<sup>23</sup> Technical dossier/1st submission/p. 25, 53; Technical dossier/1st submission/Annex 1, Annex 3; Technical dossier/2nd submission, 30 January 2015/Annex 1/LOQ: aflatoxins B1, B2, G1 and G2 < 0.2  $\mu$ g/kg, deoxynivalenol < 10  $\mu$ g/kg, HT-2 toxin < 10  $\mu$ g/kg, T-2 toxin < 10  $\mu$ g/kg, zearalenone < 10  $\mu$ g/kg, ochratoxin A < 0.5  $\mu$ g/kg and sterigmatocystin < 10  $\mu$ g/kg.

<sup>24</sup> Technical dossier/1st submission/p. 12, 16, 47.

<sup>25</sup> Technical dossier/1st submission/p. 13, 48–49; Technical dossier/1st submission/Annex 8; Technical dossier/2nd submission, 30 January 2015/Annex 3; Technical dossier/Additional data, 31 March 2021/Annex 3.

<sup>26</sup> Technical dossier/Additional data, 31 March 2021/Annex 3.

No information is available on oral and respiratory sensitisation or elicitation reactions of this  $\beta$ -galactosidase.

One study has suggested that  $\beta$ -galactosidase derived from pollen of plants is a potential allergen (Bistoni et al., 2005). In addition, two case reports describing allergic reactions (swollen throat, shortness of breath and difficulty in swallowing) following ingestion of  $\beta$ -galactosidase pills, and confirmed by antigen challenge, have been reported (Binkley, 1996; Stöcker et al., 2016; Voisin and Borici-Mazi, 2016).

Cases of occupational allergy (rhinitis, conjunctivitis, sneeze, cough, shortness of breath and pruritus) following exposure to  $\beta$ -galactosidases by inhalation or by skin and mucous membrane contact have been reported (Muir et al., 1997; Bernstein et al., 1999; Laukkanen et al., 2007; Green and Beezhold, 2011; Stöcker et al., 2016). However, several studies have shown that adults with occupational asthma to a food enzyme may be able to ingest the corresponding respiratory allergens without acquiring clinical symptoms of food allergy (Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). Moreover, these  $\beta$ -galactosidases were derived from *Aspergillus oryzae* and *Aspergillus niger*, and no homology with  $\beta$ -galactosidase from *K. lactis* strain AE-KL was reported.<sup>27</sup>

██████████, a known source of allergens, is present in the media fed to the microorganisms. However, during the fermentation process, this will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the microbial biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that potentially allergenic residues from ██████████ is 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 cannot be excluded, but the likelihood of such reactions to occur is low.

### 3.5. Dietary exposure

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

The food enzyme is intended to be used in three food processes at the recommended use levels summarised in Table 2.

**Table 2:** Intended uses and recommended use levels of the food enzyme as provided by the applicant<sup>(d)</sup>

| Food manufacturing process <sup>(a)</sup>                         | Raw material (RM)     | Recommended use level (mg TOS/kg RM) <sup>(b),(c)</sup> |
|-------------------------------------------------------------------|-----------------------|---------------------------------------------------------|
| Lactose hydrolysis in milk processing (including infant formulae) | Milk                  | 5.5– <b>60.5</b>                                        |
|                                                                   | Infant formula powder | 5.1– <b>84.7</b>                                        |
|                                                                   | Infant formula liquid | 5.5– <b>60.5</b>                                        |
| Production of fermented milk products                             | Milk                  | 5.5– <b>60.5</b>                                        |
| Manufacture of galacto-oligosaccharides                           | Lactose               | 5.3– <b>289.9</b>                                       |

TOS: total organic solids.

(a): The description has been harmonised by EFSA on the basis of additional information provided by the applicant in May 2022 and 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.

(b): Based on the mean protein content of 5.44% in three food enzyme batches used for commercialisation.

(c): The numbers in bold were used for calculation.

(d): Technical dossier/Additional data, 31 March 2021/Answer to point 4.1.

To hydrolyse lactose in milk, the food enzyme is added to milk, resulting in the release of galactose and glucose. No separation step is applied to remove the enzyme from the final foods (lactose-reduced milk and milk products).<sup>28</sup>

For the production of GOS, the food enzyme is added to lactose during the incubation step. In the presence of a high concentration of lactose,  $\beta$ -galactosidase will transglycosylate lactose resulting in

<sup>27</sup> Technical dossier/Additional data, 31 March 2021/Annex 4.

<sup>28</sup> Technical dossier/1st submission/p. 41.



the production of GOS. The  $\beta$ -galactosidase is also used to hydrolyse unreacted lactose remaining in GOS. Downstream treatment of the GOS products involves filtration and deionisation,<sup>29</sup> which are expected to remove residues of the food enzyme-TOS from the final GOS products. However, the applicant did not provide experimental data to establish the extent of the possible removal. In the absence of such information, EFSA proceeded to calculate the dietary exposure by implementing a scenario with 100% of TOS remaining in the GOS products.

Based on data provided on thermostability (see Section 3.3.1), it is expected that this  $\beta$ -galactosidase is inactivated and denatured by heat treatment during the pasteurisation step.

### 3.5.2. Dietary exposure estimation

Chronic exposure to the food enzyme-TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSA CEP Panel, 2021a). The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP Panel, 2021b). Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period (days) and normalised for body weight. This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only one day per subject were excluded and high-level exposure/intake was calculated for only those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011).

Table 3 provides an overview of the derived exposure estimates across all surveys. Detailed mean and 95th percentile exposure to the food enzyme-TOS per age class, country and survey, as well as contribution from each FoodEx category to the total dietary exposure are reported in Appendix A – Tables 1 and 2. For the present assessment, food consumption data were available from 41 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). The highest dietary exposure was estimated to be about 7.933 mg TOS/kg bw per day in infants at the 95th percentile.

**Table 3:** Summary of estimated dietary exposure to food enzyme-TOS in six population groups

| Population group                           | Estimated exposure (mg TOS/kg body weight per day) |                     |                     |                     |                     |                     |
|--------------------------------------------|----------------------------------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
|                                            | Infants                                            | Toddlers            | Children            | Adolescents         | Adults              | The elderly         |
| <b>Age range</b>                           | 3–11 months                                        | 12–35 months        | 3–9 years           | 10–17 years         | 18–64 years         | ≥ 65 years          |
| <b>Min–max mean</b><br>(number of surveys) | 0.693–4.031<br>(11)                                | 0.176–2.988<br>(15) | 0.379–1.875<br>(19) | 0.061–0.679<br>(21) | 0.065–0.276<br>(22) | 0.018–0.252<br>(22) |
| <b>Min–max 95th</b><br>(number of surveys) | 2.214–7.933<br>(9)                                 | 2.234–5.578<br>(13) | 0.957–3.129<br>(19) | 0.266–1.438<br>(20) | 0.243–0.846<br>(22) | 0.250–0.623<br>(21) |

TOS: total organic solids.

### 3.5.3. Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the following sources of uncertainties have been considered and are summarised in Table 4.

<sup>29</sup> Technical dossier/Additional data, 31 March 2021/Answer to point 4.2.

**Table 4:** Qualitative evaluation of the influence of uncertainties on the dietary exposure estimate

| Sources of uncertainties                                                                                                                                       | Direction of impact |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|
| <b>Model input data</b>                                                                                                                                        |                     |
| Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard                                              | +/-                 |
| Use of data from food consumption surveys of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)                        | +                   |
| Possible national differences in categorisation and classification of food                                                                                     | +/-                 |
| <b>Model assumptions and factors</b>                                                                                                                           |                     |
| Exposure to food enzyme-TOS was always calculated based on the recommended maximum use level                                                                   | +                   |
| Selection of broad FoodEx categories for the exposure assessment                                                                                               | +                   |
| Inclusion of semi-soft and soft cheeses that are ripened normally less than 3 months                                                                           | +                   |
| The food enzyme under application is intended for the production of GOS, however, the calculation included also other indigestible oligosaccharides (e.g. FOS) | +                   |
| Use of recipe fractions in disaggregation FoodEx categories                                                                                                    | +/-                 |
| Use of technical factors in the exposure model                                                                                                                 | +/-                 |

TOS: total organic solids; FOS: fructooligosaccharides; GOS: galacto-oligosaccharides.

+: Uncertainty with potential to cause overestimation of exposure.

-: Uncertainty with potential to cause underestimation of exposure.

The conservative approach applied to the exposure estimate to food enzyme-TOS, in particular assumptions made on the occurrence and use levels of this specific food enzyme, is likely to have led to overestimation of the exposure.

### 3.6. Margin of exposure

Given the QPS status of the production strain and the lack of hazards resulting from the food enzyme manufacturing process, toxicity tests were considered unnecessary by the Panel and the margin of exposure was not calculated.

## 4. Conclusions

Based on the data provided and the QPS status of the production strain, the Panel concluded that the food enzyme  $\beta$ -galactosidase produced with the non-genetically modified *K. lactis* strain AE-KL does not give rise to safety concerns under the intended conditions of use.

## 5. Documentation as provided to EFSA

- 1) Technical dossier 'Application for authorisation of beta-galactosidase from *Kluyveromyces lactis* AE-KL in accordance Regulation (EC) No 1331/2008'. 18 July 2014. Submitted by Amano Enzyme Inc.
- 2) Additional information. 30 January 2015. Submitted by Amano Enzyme Inc.
- 3) Additional information. 31 March 2021. Submitted by Amano Enzyme Inc.
- 4) Summary report on technical data and dietary exposure. 11 December 2015. Delivered by Hylobates Consulting and BiCT (Rome and Lodi, Italy).

## References

- Armentia A, Diaz-Perales A, Castrodeza J, Duenas-Laita A, Palacin A and Fern ~ andez S, 2009. Why can patients with baker's asthma tolerate wheat flour ingestion? Is wheat pollen allergy relevant? *Allergologia et Immunopathologia*, 37, 203–204. <https://doi.org/10.1016/j.aller.2009.05.001>
- Bernstein JA, Bernstein DI, Stauder T, Lummus Z and Bernstein IL, 1999. A cross-sectional survey of sensitization to *Aspergillus oryzae*-derived lactase in pharmaceutical workers. *The Journal of Allergy and Clinical Immunology*, 103, 1153–1157. [https://doi.org/10.1016/S0091-6749\(99\)70192-2](https://doi.org/10.1016/S0091-6749(99)70192-2)
- Binkley KE, 1996. Allergy to supplemental lactase enzyme. *The Journal of Allergy and Clinical Immunology*, 97, 1414–1416. [https://doi.org/10.1016/S0091-6749\(96\)70213-0](https://doi.org/10.1016/S0091-6749(96)70213-0)

- Bistoni O, Emiliani C, Agea E, Russano AM, Mencarelli S, Orlacchio A and Spinozzi F, 2005. Biochemical and immunological characterization of pollen-derived  $\beta$ -galactosidase reveals a new cross-reactive class of allergens among Mediterranean trees. *International Archives of Allergy and Immunology*, 136, 123–133. <https://doi.org/10.1159/000083319>
- Brisman J, 2002. Baker's asthma. *Occupational and Environmental Medicine*, 59, 498–502, quiz 502, 426.
- EFSA (European Food Safety Authority), 2006. Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment. *EFSA Journal* 2006;5(1):438, 54 pp. <https://doi.org/10.2903/j.efsa.2007.438>
- EFSA (European Food Safety Authority), 2009. Guidance of EFSA prepared by the Scientific Committee on transparency in 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 (European Food Safety Authority), 2011. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. *EFSA Journal* 2011;9(3):2097, 34 pp. <https://doi.org/10.2903/j.efsa.2011.2097>
- EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Hilbert F, Lindqvist R, Nauta M, Peixe L, Ru G, Simmons M, Skandamis P, Suffredini E, Cocconcelli PS, Fernández Escámez PS, Maradona MP, Querol A, Suarez JE, Sundh I, Vlak J, Barizzone F, Correia S and Herman L, 2020. The list of QPS status recommended biological agents for safety risk assessments carried out by EFSA. *Zenodo*. <https://doi.org/10.5281/zenodo.3828466>
- EFSA CEF Panel (EFSA Panel on Food Contact Materials, 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 Materials, Enzymes, Flavourings and Processing Aids. *EFSA Journal* 2009;7(8):1305, 26 pp. <https://doi.org/10.2903/j.efsa.2009.1305>
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), 2019. Statement on the characterisation of microorganisms used for the production of food enzymes. *EFSA Journal* 2019;17(6):5741, 13 pp. <https://doi.org/10.2903/j.efsa.2019.5741>
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré C, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mengelers M, Mortensen A, Rivière G, Steffensen I-L, Tlustos C, Van Loveren H, Vernis L, Zorn H, Glandorf B, Herman L, Aguilera J, Andryszkiewicz M, Gomes A, Kovalkovicova N, Liu Y, Rainieri S and Chesson A, 2021a. Scientific Guidance for the submission of dossiers on Food Enzymes. *EFSA Journal* 2021;19(10):6851, 37 pp. <https://doi.org/10.2903/j.efsa.2021.6851>
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré C, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mengelers M, Mortensen A, Rivière G, Steffensen I-L, Tlustos C, van Loveren H, Vernis L, Zorn H, Liu Y and Chesson A, 2021b. Statement on the process-specific technical data used in exposure assessment of food enzymes. *EFSA Journal* 2021;19(12):7010, 38 pp. <https://doi.org/10.2903/j.efsa.2021.7010>
- 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 Nations/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, 63–67. Available online: <https://www.fao.org/3/a0675e/a0675e.pdf>
- Green BJ and Beezhold DH, 2011. Industrial fungal enzymes: an occupational allergen perspective. *Journal of Allergy*, 682574. <https://doi.org/10.1155/2011/682574>
- Laukkanen A, Ruoppi P, Remes S, Koistinen T and Mäkinen-Kiljunen S, 2007. Lactase-induced occupational protein contact dermatitis and allergic rhinoconjunctivitis. *Contact Dermatitis*, 57, 89–93. <https://doi.org/10.1111/j.1600-0536.2007.01158.x>
- Muir DC, Verrall AB, Julian JA, Millman JM, Beaudin MA and Dolovich J, 1997. Occupational sensitization to lactase. *American Journal of Industrial Medicine*, 31, 570–571. [https://doi.org/10.1002/\(sici\)1097-0274\(199705\)31:5<570::aid-ajim11>3.0.co;2-w](https://doi.org/10.1002/(sici)1097-0274(199705)31:5<570::aid-ajim11>3.0.co;2-w)
- Poulsen LK, 2004. Allergy assessment of foods or ingredients derived from biotechnology, gene-modified organisms, or novel foods. *Molecular Nutrition and Food Research*, 48, 413–423. <https://doi.org/10.1002/mnfr.200400029>
- Stöcker B, Grundmann S, Mosters P, Nitzsche P and Brehler R, 2016. Occupational sensitization to lactase in the dietary supplement industry. *Archives of Environmental and Occupational Health*, 71, 259–267. <https://doi.org/10.1080/19338244.2015.1066294>
- Voisin MR and Borici-Mazi R, 2016. Anaphylaxis to supplemental oral lactase enzyme. *Allergy, Asthma & Clinical Immunology*, 12, 66. Available online: <https://aacijournal.biomedcentral.com/articles/10.1186/s13223-016-0171-8>

## Abbreviations

|                   |                                                                                |
|-------------------|--------------------------------------------------------------------------------|
| bw                | body weight                                                                    |
| CAS               | Chemical Abstracts Service                                                     |
| EFSA BIOHAZ Panel | EFSA Panel on Biological Hazards                                               |
| EFSA CEF Panel    | EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids |
| EFSA CEP Panel    | EFSA Panel on Food Contact Materials, Enzymes and Processing Aids              |
| EFSA GMO Panel    | EFSA Panel on genetically modified organisms                                   |
| EINECS            | European Inventory of Existing Commercial Chemical Substances                  |
| FAO               | Food and Agricultural Organization of the United Nations                       |
| FoodEx            | a standardised food classification and description system                      |
| FOS               | fructooligosaccharides                                                         |
| GM                | genetically modified                                                           |
| GMO               | genetically modified organism                                                  |
| GOS               | galactooligosaccharides                                                        |
| IUBMB             | International Union of Biochemistry and Molecular Biology                      |
| JECFA             | Joint FAO/WHO Expert Committee on Food Additives                               |
| LOQ               | limit of quantification                                                        |
| █                 | █                                                                              |
| non-GM            | non-genetically modified                                                       |
| QPS               | Qualified Presumption of Safety                                                |
| RM                | raw material                                                                   |
| SDS-PAGE          | sodium dodecyl sulfate-polyacrylamide gel electrophoresis                      |
| TOS               | total organic solids                                                           |
| U                 | unit                                                                           |
| WHO               | World Health Organization                                                      |

## Appendix A – Dietary exposure estimates to the food enzyme–TOS in details

Information provided in this appendix is shown in an excel file (downloadable <https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2022.7571#support-information-section>).

The file contains two sheets, corresponding to two tables.

Table 1: Mean and 95th percentile exposure to the food enzyme–TOS per age class, country and survey

Table 2: Contribution of food categories to the dietary exposure to the food enzyme–TOS per age class, country and survey

## Appendix B – Population groups considered for the exposure assessment

| Population                       | Age range                                             | Countries with food consumption surveys covering more than one day                                                                                                                              |
|----------------------------------|-------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Infants</b>                   | From 12 weeks on up to and including 11 months of age | Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia                                                                                                 |
| <b>Toddlers</b>                  | From 12 months up to and including 35 months of age   | Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Netherlands, Portugal, Slovenia, Spain                                                           |
| <b>Children</b>                  | From 36 months up to and including 9 years of age     | Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Spain, Sweden                            |
| <b>Adolescents</b>               | From 10 years up to and including 17 years of age     | Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden                   |
| <b>Adults</b>                    | From 18 years up to and including 64 years of age     | Austria, Belgium, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden |
| <b>The elderly<sup>(a)</sup></b> | From 65 years of age and older                        | Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden                          |

(a): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011).