Safety evaluation of the food enzyme glucose isomerase from *Streptomyces murinus* (strain NZYM-GA)

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**Abstract**

The food enzyme is a glucose isomerase (α-xylose aldose-ketose-isomerase; EC 5.3.1.5) produced with a non-genetically modified *Streptomyces murinus* strain NZYM-GA by Novozymes A/S. The glucose isomerase is intended only to be used in an immobilised form in glucose isomerisation for the production of high fructose syrups. Residual amounts of total organic solids are removed by the purification steps applied during the production of high fructose syrups using the immobilised enzyme; consequently, dietary exposure was not calculated. Genotoxicity tests did not raise a safety concern. Similarity of the amino acid sequence to those of known allergens was searched and no match was found. The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but the likelihood to occur is considered to be low. Based on the data provided, the immobilisation process and the removal of total organic solids during the production of high fructose syrups, 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, glucose isomerase, xylose isomerase, α-xylose aldose-ketose-isomerase, EC 5.3.1.5, *Streptomyces murinus*

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1. **Introduction**

Article 3 of the Regulation (EC) No 1332/2008\(^1\) 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\(^2\) established the European Union (EU) procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

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

All food enzymes currently on the 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 companies "Danisco US Inc." for the authorisation of the food enzyme Mucorpepsin from *Rhizomucor miehei*, "Novozymes A/S" for the authorisation of the food enzymes Acetolactate decarboxylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-JB) and Glucose isomerase from *Streptomyces murinus* (strain NZYM-GA), and "Amano Enzyme Inc." for the authorisation of the food enzymes 4-alpha-glucanotransferase from *Geobacillus pallidus* (strain AE-SAS) and Tannase from *Aspergillus niger* (strain AE-TAN).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011\(^3\) 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 11 of that Regulation.

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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, p. 7–15.

\(^2\) Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, 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.
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 Mucorpepsin from *Rhizomucor miehei*, Acetolactate decarboxylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-JB), Glucose isomerase from *Streptomyces murinus* (strain NZYM-GA), 4-alpha-glucanotransferase from *Geobacillus pallidus* (strain AE-SAS) and Tannase from *Aspergillus niger* (strain AE-TAN) 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 food enzyme glucose isomerase from *S. murinus* strain NZYM-GA.

1.3. Information on existing authorisations and evaluations

The applicant reports that the French and Danish authorities have evaluated and authorised the use of glucose isomerase from *S. murinus* in fructose syrup production. The Danish authority also sets out the conditions of use, including the dosages for specific foods, which is up to 30 Immobilised Glucose Isomerase Units (IGIU)/kg syrup.4

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier on support of the application for authorisation of the food enzyme glucose isomerase obtained from *S. murinus* strain NZYM-GA.

Additional information was sought from the applicant during the assessment process in two requests from EFSA sent on 11 April 2017 and 23 February 2018, and was consequently provided (see 'Documentation provided to EFSA').

Following the request for additional data sent by EFSA on 23 February 2018, the applicant requested a clarification teleconference, which was held on 19 April 2018.

2.2. Methodologies

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

The current ‘Guidance on the submission of a dossier on food enzymes for safety evaluation’ (EFSA CEF Panel, 2009) has been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance to the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016).

3. Assessment

**IUBMB nomenclature:** Xylose isomerase  
**Systematic name:** D-xylose aldose-ketose-isomerase  
**Synonyms:** Glucose isomerase; D-xylose isomerase; D-xylose ketol-isomerase  
**IUBMB No:** EC 5.3.1.5  
**CAS No:** 9023-82-9  
**EINECS No:** 232-944-6.

Xylose isomerase catalyses the conversion of D-xylose to D-xylulose and of D-glucose to D-fructose.5 It requires Mg2+ as a co-factor.6 It is intended to be used in glucose isomerisation for the production of high fructose syrups. Based on its technical application, the term glucose isomerase is used throughout this dossier. The food enzyme is intended to be used only in an immobilised form.
3.1. Source of the food enzyme

The glucose isomerase is produced with the bacterium *S. murinus* strain NZYM-GA, which is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Germany, DSMZ) with the deposit number DSM 3252, which was isolated from a soil sample from Venezuela. The production strain showed 100% identity to *Streptomyces costaricanus*, *Streptomyces graminearus*, *Streptomyces griseofuscus* and *Streptomyces murinus* based on 16S rRNA analysis. In conventional carbon utilisation tests, the production strain showed the same behaviour as *S. murinus*. Furthermore, the parental strain DSM 3252 was identified as *S. murinus* by extensive physiological and chemotaxonomical data.

It has been shown that *S. murinus* DSM 3252 does not produce any pentaene or polyene antibiotics or any other compounds which exhibit antimicrobial activity (Ashby et al., 1987). No experimental data has been provided by the applicant on the absence of potential antibiotic resistance genes in the production strain.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004, with food safety procedures based on Hazard Analysis and Critical Control Points (HACCP), and in accordance with current Good Manufacturing Practice (GMP).

The production strain is grown as a pure culture using a typical industrial medium in a submerged, fed-batch fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration leaving a supernatant containing the food enzyme. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step, in which enzyme protein is retained while 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

The glucose isomerase is a polypeptide chain of 388 amino acids. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis consistently showed one major protein band in all batches migrating below the 45-kDa reference protein. The food enzyme was tested for α-amylase, glucoamylase, lipase and protease activities and no relevant activities were detected. No other enzymatic side activities were reported.

The in-house determination of glucose isomerase activity is based on the conversion of fructose to glucose (reaction conditions: 37°C, pH 7.0, 5 min). Glucose is then oxidised in the presence of glucose oxidase, releasing hydrogen peroxide as a side product, which is used as co-substrate by a peroxidase to oxidise 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (reaction conditions: 37°C, pH 7.0, 8 min). The enzymatic activity is determined by measuring the amount of formed ABTS** spectrophotometrically at 405 nm. The enzyme activity is measured relative to an internal enzyme standard, and is expressed in Fructose Isomerase Novo Units, Total (FINU(T))/g. For the immobilised enzyme, the glucose isomerase activity is expressed in IGIU/g. The quantification is based on fructose formation from glucose, and determination by polarimetry (reaction conditions: 60°C, pH 7.5).
The food enzyme has been characterised with regard to its temperature and pH profiles. The temperature profile has been measured from 50°C up to 64°C, with a maximum at 64°C (pH 7.5), and the pH profile has been measured within a pH range of 7.1–7.9, with a maximum at pH 7.9 (60°C).15

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme have been provided for four food enzyme batches, three batches used for commercialisation and one batch used for the toxicological tests (Table 1).16 The average total organic solids (TOS) content of the three commercial batches was 10.2% (range 9.9–10.4%). The average enzyme activity/TOS ratio of the three batches for commercialisation is 11.26 FINU(T)/mg TOS.

Table 1: Compositional data of the food enzyme

| Parameter                        | Unit                  | Batches          |
|----------------------------------|-----------------------|------------------|
| Glucose isomerase activity       | FINU(T)/g batch(b)    | 885              |
|                                  |                       | 1,290            |
|                                  |                       | 1,280            |
|                                  |                       | 1,130            |
| Protein                          | %                     | 2.3              |
|                                  |                       | 3.3              |
|                                  |                       | 3.3              |
|                                  |                       | 3.0              |
| Ash                              | %                     | 2.9              |
|                                  |                       | 1.8              |
|                                  |                       | 2.2              |
|                                  |                       | 2.2              |
| Water                            | %                     | 87.2             |
|                                  |                       | 87.9             |
|                                  |                       | 87.4             |
|                                  |                       | 88.1             |
| Total organic solids (TOS)(c)    | %                     | 9.9              |
|                                  |                       | 10.3             |
|                                  |                       | 10.4             |
|                                  |                       | 9.7              |
| Glucose isomerase activity/mg TOS| FINU(T)/mg TOS        | 8.94             |
|                                  |                       | 12.52            |
|                                  |                       | 12.31            |
|                                  |                       | 11.6             |

(a): Batch used for the bacterial reverse mutation and in vitro mammalian cell micronucleus tests.
(b): FINU(T): Fructose Isomerase Novo Units, Total (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 4 used for genotoxicity studies was below 0.5 mg/kg which complies with the specification for lead (≤ 5 mg/kg) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006). In addition, the levels of arsenic, cadmium and mercury were below the limits of detection (LODs) of the employed methodologies.17,18

The food enzyme complies with the microbiological criteria as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006), which stipulate that Escherichia coli and Salmonella species are absent in 25 g of sample, and total coliforms are not more than 30 colony forming units per gram.19 No antimicrobial activity was detected in any of these batches (FAO/WHO, 2006).19

3.3.4. Viable cells of the production strain

The absence of viable cells of the production strain in the food enzyme was shown in three independent production batches using non-selective culturing at 30–35°C for 72 h.20

3.4. Toxicological data

The applicant provided a bacterial gene mutation assay (Ames test), and an in vitro mammalian cell micronucleus test performed with the food enzyme under assessment (batch 4, Table 1). The applicant also provided a repeated dose 90-day oral toxicity study performed with a glucose isomerase produced using a previous production strain that had not yet been subjected to classical mutagenesis for yield enhancement.21 This study was not considered, as it could not be ascertained that the test item was fully representative of the food enzyme under assessment.

15 Technical dossier/p. 38–39 and Annex 7.
16 Technical dossier/p. 31, p. 59 and Additional data July 17.
17 LOD: Pb = 0.5 mg/kg; As = 0.1 mg/kg; Cd = 0.05 mg/kg; Hg = 0.03 mg/kg.
18 Technical dossier/p. 33 and Additional data July 2017.
19 Technical dossier/p. 35 and Additional data July 2017.
20 Technical dossier/Annex 1.07 and Additional data July 2017.
21 Technical dossier/p. 58.
3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay was performed according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997) and following Good Laboratory Practice (GLP). Four strains of *Salmonella* Typhimurium (TA1535, TA100, TA1537 and TA98) and *Escherichia coli* strain WP2uvrA pKM101 were tested in the presence or absence of metabolic activation applying the 'treat and plate assay'. Two independent experiments were carried out using six different concentrations of the food enzyme (156, 313, 625, 1,250, 2,500 and 5,000 μg food enzyme dry matter/mL, corresponding to 127, 255, 510, 1,019, 2,038 and 4,076 μg TOS/mL). No significant cytotoxicity was observed at any concentration level of the test substance, based on viable cells counts. Upon treatment with the food enzyme, there was no significant increase in revertant colony numbers above the control values in any strain with or without S9-mix.

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

3.4.1.2. *In vitro* mammalian cell micronucleus test

The *in vitro* micronucleus test was carried out according to the OECD Test Guideline 487 (OECD, 2010) and following GLP. Two separate experiments were performed in duplicate cultures of human peripheral whole blood lymphocytes. Cells were exposed to the test substance for 3 h in the presence or absence of the S9-mix and harvested 24 h after the beginning of the treatment (3 + 21 h treatment). Additionally, a continuous 24-h treatment without S9-mix was included with harvesting 24 h after removal of the test substance (24 + 24 h treatment). The food enzyme was tested at 3,000, 4,000 and 5,000 food enzyme μg/mL, corresponding to 291, 388 and 485 μg TOS/mL. Cytotoxicity did not exceed 15% of concurrent negative control values after treatment, both in the presence and absence of S9-mix. The frequency of binucleated cells with micronuclei (MNBN) was comparable to the negative controls at all doses tested; exception was the 3 + 21 h treatment in the presence of S9-mix at 4,000 μg/mL, where a statistically significant increase in MNBN cells was observed. However, the Panel noted that these values were within the 95% of the historical control range and were considered not biologically relevant. The Panel concluded that the food enzyme glucose isomerase did not induce an increase in the frequency of MNBNs in cultured human peripheral blood lymphocytes, under the test conditions employed in this study.

3.4.2. Allergenicity

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

The potential allergenicity of the glucose isomerase produced with the non-genetically modified *S. murinus* strain NZYM-GA was assessed by comparing its amino acid sequence with those of known allergens according to the scientific opinion on the assessment of allergenicity of genetically modified 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.7

No information is available on oral sensitisation or elicitation reactions of this glucose isomerase. In addition, no allergic reactions upon dietary exposure to any glucose isomerase have been reported in the literature. Therefore, it can be concluded that the likelihood of an allergic reaction upon oral ingestion of this glucose isomerase, produced with the non-genetically modified *S. murinus* strain NZYM-GA, cannot be excluded, but the likelihood of such a reaction to occur is considered to be low.

Quantifying the risk for allergenicity is not possible in view of the individual susceptibility to food allergens. Allergenicity can be ruled out only if the proteins are fully removed. Considering that the food enzyme is only used in immobilised form7 and in glucose isomerisation for the production of high fructose syrups, experimental data showed a significant removal (below LODs) of protein. However, traces of protein could be present in high fructose syrups.

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22 Technical dossier/Annex 6.01.
23 Technical dossier/Annex 6.02.
The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme cannot be excluded but the likelihood of such reactions occurring is considered to be low.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in glucose isomerisation for the production of high fructose syrups. The applicant states that the food enzyme is only intended to be used in an immobilised form. The glucose isomerase enzyme is immobilised. As the food enzyme is intended to be used only in its immobilised form, the transfer of TOS into the final product, i.e. high fructose syrups, is expected to be negligible. Additionally, experimental data have been provided showing low ash contents (less than 0.01 g/100 g dry matter syrup), and protein, fat and fibres contents in the final high fructose syrup, after purification steps are applied (i.e. ion exchange chromatography, treatment with active carbon), below the LODs using methods in accordance with Regulation (EU) 1169/2011 (Documentation provided to EFSA No 5). Amounts of TOS in the final food samples have been experimentally shown to be not detectable. The Panel considers that the residual amount of TOS (including substances other than proteins, such as DNA fragments) in the final high fructose syrups will be removed.

3.5.2. Dietary exposure estimation

Considering that the food enzyme is intended to be used only in its immobilised form (see Section 3.5.1), and as residual amounts of TOS are removed by the purification steps applied during the production of high fructose syrups, a dietary exposure was not calculated.

4. Conclusions

Based on the data provided, immobilisation of the food enzyme and the removal of TOS during purification steps applied during the production of high fructose syrups, the Panel concluded that the food enzyme glucose isomerase produced with the S. murinus strain NZYM-GA does not give rise to safety concerns under the intended conditions of use.

Documentation provided to EFSA

1) Technical dossier ‘Glucose isomerase produced by a strain of Streptomyces murinus (strain NZYM-GA)’. March 2015. Submitted by Novozymes A/S.
2) Summary report on technical data and dietary exposure related to glucose isomerase from Streptomyces murinus (strain NZYM-GA) by Novozymes. November 2016. Delivered by Hylobates Consulting (Rome, Italy) and BiCT (Lodi, Italy).
3) Additional information. July 2017. Submitted by Novozymes A/S.
4) Additional information. July 2018. Submitted by Novozymes A/S.
5) Additional information on the transfer of food enzymes into food for the food processes refined sugar production and processing. September 2017. Provided by the Association of Manufacturers and Formulators of Enzyme Products.

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OECD (Organisation for Economic Co-Operation and Development), 2010. OECD Guideline for the Testing of Chemicals. In Vitro Mammalian Cell Micronucleus Test. No. 487. 22 July 2010. 29 pp. Available online: https://www.oecd-ilibrary.org/environment/test-no-487-in-vitro-mammalian-cell-micronucleus-test_9789264091016-en

Abbreviations

ABTS 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid
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 Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
EC Enzyme Commission
EINECS European Inventory of Existing Commercial Chemical Substances
FAO Food and Agricultural Organisation
FINU(T) Fructose Isomerase Novo Unit, Total
GLP Good Laboratory Practice
GMO Genetically Modified Organisms
GMP Good Manufacturing Practice
HACCP Hazard Analysis and Critical Control Points
IGIU Immobilised Glucose Isomerase Units
IUBMB International Union of Biochemistry and Molecular Biology
JECA Joint FAO/WHO Expert Committee on Food Additives
LOD limit of detection
MNBN binucleated cells with micronuclei
OECD Organisation for Economic Cooperation and Development
rRNA ribossomal ribonucleic acid
SDS-PAGE sodium dodecyl sulfate-poly acrylamide gel electrophoresis
TOS total organic solids
WHO World Health Organization