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Safety evaluation of the food enzyme glucan 1,4-α-maltohydrolase from the genetically modified *Bacillus licheniformis* strain NZYM-SD

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Abstract

The food enzyme glucan 1,4-α-maltohydrolase (4-α-D-glucan α-maltohydrolase; 3.2.1.133) is produced with the genetically modified *Bacillus licheniformis* strain NZYM-SD by Novozymes A/S. The genetic modifications did not give rise to safety concerns. The production strain has been shown to qualify for Qualified Presumption of Safety (QPS) status. The food enzyme is free from viable cells of the production organism and its DNA. The food enzyme is intended to be used in three food manufacturing processes, namely baking processes and brewing processes and starch processing for glucose syrup production and other starch hydrolysates. Since residual amounts of total organic solids (TOS) are removed by the purification steps applied during the production of glucose syrups, dietary exposure was calculated only for baking and brewing processes. Dietary exposure was estimated to be up to 0.57 mg TOS/kg body weight (bw) per day in European populations. Given the QPS status of the production strain and the lack of hazards resulting from the food enzyme manufacturing process, toxicological studies were not considered necessary. Similarity of the amino acid sequence to those of known allergens was searched and four matches were found. 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 considered to be low. Based on the data provided, the QPS status of the production strain and the absence of issues arising from the production process, the Panel concluded that the food enzyme glucan 1,4-α-maltohydrolase produced with the genetically modified *B. licheniformis* strain NZYM-SD does not give rise to safety concerns under the intended conditions of use.

Keywords: Food enzyme, glucan 1,4-α-maltohydrolase, 4-alpha-D-glucan alpha-maltohydrolase, maltogenic amylase, 3.2.1.133, *Bacillus licheniformis*, genetically modified microorganism

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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:

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

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

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

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

1.1.1. Background as provided by the European Commission

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

An application has been introduced by the applicant "Novozymes A/S" for the authorization of food enzyme Maltogenic amylase from a genetically modified *Bacillus licheniformis* (strain NZYM-SD).

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 application falls 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

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002[4], the European Commission requests the European Food Safety Authority to carry out the safety assessment on the following food enzyme: Maltogenic amylase from a genetically modified *Bacillus licheniformis* (strain NZYM-SD), in

[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, pp. 15–24.

[4] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, pp. 1–24.

accordance with the Regulation (EC) No 1331/2008 establishing a common authorization procedure for food additives, food enzymes and food flavourings.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme maltogenic amylase from a genetically modified *B. licheniformis* (strain NZYM-SD). The dossier was updated on 21 May 2021.

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

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA 'Guidance on transparency in the scientific aspects of risk assessment' (EFSA, 2009b) as well as in the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) and following the relevant existing guidances of EFSA Scientific Committees.

The current 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) has been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance with the methodology described in the CEF Panel 'Statement on the exposure assessment of food enzymes' (EFSA CEP Panel, 2021a).

3. Assessment

IUBMB nomenclature	Glucan 1,4-α-maltohydrolase
Systematic name	4-α-D-glucan α-maltohydrolase
Synonyms	Maltogenic amylase
IUBMB No	EC 3.2.1.133
CAS No	160611-47-2
EINECS No	630-523-5

Glucan 1,4-α-maltohydrolases catalyse the hydrolysis of (1→4)-α-D-glucosidic linkages in starch polysaccharides, to successively release maltose units from the non-reducing chain ends. The food enzyme is intended to be used in three food manufacturing processes, namely baking processes, brewing processes and starch processing for production of glucose syrup and other starch hydrolysates.

3.1. Source of the food enzyme

The glucan 1,4-α-maltohydrolase is produced with the genetically modified bacterium *B. licheniformis* strain NZYM-SD, which is deposited at the German Collection of Microorganisms and Cell Cultures (DSMZ, Germany), with deposit number [redacted][5]

The production strain was identified as *B. licheniformis* [redacted][6]

The species *B. licheniformis* is included in the list of organisms for which the qualified presumption of safety (QPS) may be applied, provided that the absence of acquired antimicrobial resistance (AMR) genes and toxigenic activity are verified for the specific strain used (EFSA BIOHAZ Panel, 2021). [redacted][8]

[5] Technical dossier/2nd submission/Annex A2.
[6] Technical dossier/2nd submission/Annex A1.
[7] Technical dossier/2nd submission/Annex A4.
[8] Technical dossier/2nd submission/Annex 4/p. 6.

3.1.1. Characteristics of the parental and recipient microorganisms

The parental strain [redacted]

The recipient strain [redacted][9]

3.1.2. Characteristics of introduced sequences

[redacted]

3.1.3. Description of the genetic modification process

The purpose of genetic modification was to enable the production strain to synthesise glucan 1,4-α-maltohydrolase [redacted][10]

[9] Technical dossier/2nd submission/Annexes: C1–C12.
[10] Technical dossier/2nd submission/Annexes: C1, C3 and C12.

3.1.4. Safety aspects of the genetic modification

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

[redacted][8]

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

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[11], with food safety procedures based on hazard analysis and critical control points, and in accordance with current good manufacturing practice.[12]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, [redacted] 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 further purified and concentrated, including an ultrafiltration step in which enzyme protein is retained, while most of the low molecular mass material passes the filtration membrane and is discarded.[13] 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.[14]

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

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The glucan 1,4-α-maltohydrolase is a single polypeptide chain of [redacted] amino acids.[15] The molecular mass of the mature protein, calculated from the amino acid sequence is [redacted] kDa.[15] 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 gel showed one major protein band migrating around [redacted] kDa.[16] The food enzyme was tested for glucoamylase, lipase and protease activities, and none were detected.[17] No other enzymatic activities were reported.

The in-house determination of glucan 1,4-α-maltohydrolase activity is based on hydrolysis of maltotriose to maltose and glucose (reaction conditions: pH 5.0, 37°C, 30 min). Glucose is quantified using a glucose hexokinase assay. Enzyme activity is expressed in Sweet Dough Maltogenic Units/g (SDMU/g). One SDMU corresponds to the amount of enzyme, which catalyses the hydrolysis of 100 μmol maltotriose per minute under the conditions of the assay.[18]

The food enzyme has a temperature optimum around 60°C (pH 5.5) and a pH optimum at pH 5.0 (30°C). Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures (pH 5.5). Enzyme activity decreased by 50% at 80°C, showing no activity at 85°C.[19]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (Table 1).[20] The mean total organic solids (TOS) of the three food enzyme batches is 4.1% and the mean enzyme activity/TOS ratio is 2.1 SDMU/mg TOS.

[11] 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.
[12] Technical dossier/2nd submission/p. 45/Annex 5.
[13] Technical dossier/2nd submission/p. 45–52.
[14] Technical dossier/2nd submission/Annex 6.
[15] Technical dossier/2nd submission/p. 31/Annex 1.
[16] Technical dossier/2nd submission/p. 33.
[17] Technical dossier/2nd submission/p. 38–39/Annexes: 3.02, 3.03, 3.04.
[18] Technical dossier/2nd submission/p. 35–37/Annex 3.01.
[19] Technical dossier/2nd submission/p. 37–38/Annex 8.
[20] Technical dossier/2nd submission/p. 32/Annexes: 2, 3, 9.

Table 1: Composition of the food enzyme

Parameters	Unit	Batches		
		1	2	3
Glucan 1,4-α-maltohydrolase	SDMU/g[(a)]	87.4	83.1	92.3
Protein	%	4.4	4.4	4.7
Ash	%	0.3	0.5	0.5
Water	%	95.3	95.2	95.8
Total organic solids (TOS)[(b)]	%	4.4	4.3	3.7
Activity/mg TOS	SDMU/mg TOS	2.0	1.9	2.5

(a): SDMU: Sweet Dough Maltogenic Unit, Unit/g (see Section 3.3.1).
(b): TOS calculated as 100% – % water – % ash.

3.3.3. Purity

The lead content in the three batches was below 0.5 mg/kg[21] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). In addition, the levels of arsenic, cadmium and mercury were below the limits of detection of the employed methodologies.[22,23]

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).[24] No antimicrobial activity was detected in any of the tested batches (FAO/WHO, 2006).[25]

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

3.3.4. Viable cells and DNA of the production strain

The absence of viable cells of the production strain in the food enzyme was demonstrated [redacted] No colonies were produced.[26]

The absence of recombinant DNA in the food enzyme was demonstrated [redacted] No DNA was detected [redacted][27]

3.4. Toxicological data

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

3.4.1. 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 glucan 1,4-α-maltohydrolase with the genetically modified *B. licheniformis* strain NZYM-SD 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, four matches were found. The matching allergens were Asp o 21, an α-amylase

[21] Technical dossier/2nd submission/p. 33/Annexes: 2.04 and 9.
[22] Technical dossier/2nd submission/p. 35/Annexes: 2.04 and 9.
[23] LoDs: Pb = 0.5 mg/kg, As = 0.3 mg/kg, Cd, Hg = 0.05 mg/kg each.
[24] Technical dossier/2nd submission/p. 35/Annexes: 2.07, 2.08, 2.09, 2.10 and 9.
[25] Technical dossier/2nd submission/p. 33/Annexes: 2.06 and 9.
[26] Technical dossier/2nd submission/Annex D1.
[27] Technical dossier/2nd submission/Annex D2.

from *Aspergillus oryzae*, Asp f 13, a serine-protease from *Aspergillus fumigatus*, Sch c 1, a glucoamylase from *Schizophyllum commune* and Aed a 4, an α-glucosidase from *Aedes aegypti* (yellow fever mosquito).[28]

No information is available on oral and respiratory sensitisation or elicitation reactions of this glucan 1,4-α-maltohydrolase. In addition, no allergic reactions upon dietary exposure to any glucan 1,4-α-maltohydrolase have been reported in the literature.

α-Amylase from *A. oryzae* (Brisman and Belin, 1991; Quirce et al., 1992, 2002; Sander et al., 1998; Brisman, 2002), serine protease from *A. fumigatus* (Kurup et al., 2002) and glucoamylase from *S. commune* (Toyotome et al., 2014) are known as occupational respiratory allergens associated with asthma. However, several studies have shown that adults with occupational asthma to a food enzyme (as described for α-amylase from *A. oryzae*) can ingest respiratory allergens without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Poulsen, 2004; Armentia et al., 2009). Taking into account the wide use of α-amylase as a food enzyme, only a low number of case reports has been described in literature that focused on allergic reactions upon oral exposure to α-amylase in individuals respiratory sensitised to α-amylase (Losada et al., 1992; Quirce et al., 1992; Baur and Czuppon, 1995; Kanny and Moneret-Vautrin, 1995; Moreno-Ancillo et al., 2004). Such information has not been reported for glucoamylase and serine protease. α-Glucosidase has been associated with allergic reactions to yellow fever mosquito bites, but allergic reactions upon oral exposure to the α-glucosidase from the yellow fever mosquito have not been reported. No allergic reactions upon dietary exposure to any α-glucosidase have been reported in the literature.

A product that may cause allergies or intolerances (listed in the Regulation (EU) No 1169/2011[29]) is used as raw material ([redacted]). However, during the fermentation process, this product 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 of these materials employed as protein sources are not expected to be present in the food enzyme.

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 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 three food manufacturing 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[30]

Food manufacturing process[(a)]	Raw material	Recommended use level[(b),(c)]
Baking processes	Flour	4.8–**47.6** mg TOS/kg flour
Brewing processes	Cereals (malted or not)	23.8–**69.0** mg TOS/kg cereals
Starch processing for glucose syrup production and other starch hydrolysates	Starch	2.4–19.0 mg TOS/kg starch

TOS: total organic solids.
(a): The description has been harmonised according to the 'EC working document describing the food processes in which food enzymes are intended to be used' – not yet published at the time of adoption of this opinion.
(b): Based on 2.1 SDMU/mg TOS.
(c): Numbers in bold were used for calculations.

[28] Technical dossier/2nd submission/p. 60–63/Annexes: 7.01 and 7.02.
[29] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.
[30] Technical dossier/2nd submission/p. 56–58.

In baking processes, food enzyme is added to the raw materials during the preparation of the dough.[31] It is used to shorten the branched part of the amylopectin molecules during dough handling, reducing the tendency to crystalise. The food enzyme–TOS remains in the dough.

In brewing processes, the food enzyme is added at the mashing step, resulting in improved yields due to release of maltose, decreased production time and offering the option of a wider choice of raw materials. The food enzyme–TOS remains in the beer.[32]

Based on data provided on thermostability (see Section 3.3.1), it is expected that the enzyme is inactivated during baking and brewing processes.

In starch processing, the food enzyme is added during the saccharification step.[33] The hydrolysis of starch liberates maltose and glucose. The food enzyme–TOS is removed in the final processed syrups and other starch hydrolysates by treatment with activated charcoal or similar and with ion-exchange resins (EFSA CEP Panel, 2021b).

3.5.2. Dietary exposure estimation

In accordance with the guidance document (EFSA CEP Panel, 2021a), a dietary exposure was calculated only for food manufacturing processes where the food enzyme–TOS remains in the final foods, namely baking and brewing processes.

Chronic exposure was calculated by combining the maximum recommended use level with the relevant FoodEx categories (EFSA CEP Panel, 2021b) and individual consumption data. 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 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). The highest dietary exposure to the food enzyme–TOS was estimated to be about 0.5 mg TOS/kg body weight (bw) per day in children below 10 years of age and in adults.

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**
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0.009–0.138 (11)	0.102–0.298 (15)	0.116–0.285 (19)	0.066–0.173 (21)	0.060–0.174 (22)	0.057–0.115 (22)
Min–max 95th percentile (number of surveys)	0.052–0.566 (9)	0.252–0.523 (13)	0.224–0.535 (19)	0.140–0.363 (20)	0.140–0.474 (22)	0.119–0.238 (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.

[31] Technical dossier/2nd submission/p. 71–73.
[32] Technical dossier/2nd submission/p. 73–75.
[33] Technical dossier/2nd submission/p. 76–77.

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

Sources of uncertainties	Direction of impact
Model input data	
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	+/–
Model assumptions and factors	
FoodEx categories included in the exposure assessment were assumed to always contain the food enzyme–TOS	+
Exposure to food enzyme–TOS was always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
Use of recipe fractions in disaggregation FoodEx categories	+/–
Use of technical factors in the exposure model	+/–
Exclusion of some processes from the exposure assessment – Starch processing for the production of glucose syrups and other starch hydrolysates	–

TOS: total organic solids.
+: 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.

The exclusion of one food manufacturing process (starch processing for the production of glucose syrups and other starch hydrolysates) from the exposure assessment was based on > 99% of TOS removal during these processes and is not expected to have an impact on the overall estimate derived.

3.6. Margin of exposure

Since this food enzyme qualifies for the QPS approach and since the manufacturing process raised no issue of concern, toxicological tests are considered unnecessary by the Panel. In the absence of toxicological tests, the margin of exposure was not calculated.

4. Conclusion

Based on the data provided, the QPS status of the production strain and the absence of issues arising from the production process, the Panel concluded that the food enzyme glucan 1,4-α-maltohydrolase produced with the genetically modified *B. licheniformis* strain NZYM-SD does not give rise to safety concerns under the intended conditions of use.

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

5. Documentation as provided to EFSA

Application for authorisation Maltogenic amylase produced by a genetically modified strain of *Bacillus licheniformis* (strain NZYM-SD). May 2021. Submitted by Novozymes A/S.

Additional information. March 2022. Submitted by Novozymes A/S.

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Abbreviations

ANI	average nucleotide identity
Bw	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GLP	good laboratory practice
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LoD	limit of detection
PCR	polymerase chain reaction
QPS	Qualified Presumption of Safety
SDMU	Sweet Dough Maltogenic Unit
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	total organic solids
WGS	whole genome sequence
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.2022.7368#support-information-section).

The file contains two sheets, corresponding to two tables.

Table 1: Average 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
Infants	From 12 weeks on up to and including 11 months of age	Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia
Toddlers	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
Children	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
Adolescents	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
Adults	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
The elderly[a]	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).