Adopted: 25 October 2023

DOI: 10.2903/j.efsa.2023.8410

efsa JOURNAL

SCIENTIFIC OPINION

# Safety evaluation of the food enzyme glucan 1,4-α-maltohydrolase from the genetically modified *Bacillus subtilis* strain MAMDSM

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## Abstract

The food enzyme glucan 1,4-α-maltohydrolase (4-α-D-glucan α-maltohydrolase; EC 3.2.1.133) is produced with the genetically modified *Bacillus subtilis* strain MAMDSM by DSM Food Specialties. The genetic modifications do not give rise to safety concerns. The food enzyme is free from viable cells of the production organism and its DNA. It is intended to be used in the processing of cereals and other grains for the production of baked and brewed products. Dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.204 mg TOS/kg body weight (bw) per day in European populations. The production strain meets the requirements for the QPS approach. As no concerns arising from the manufacturing process have been identified, the Panel considered that toxicological tests were not needed for the assessment of this food enzyme. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and four matches were found. The Panel considered that the risk of allergic reactions by dietary exposure cannot be excluded, but the likelihood is low. Based on the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns, under the intended conditions of use.

**KEYWORDS**

4-α-D-glucan α-maltohydrolase, *Bacillus subtilis*, EC 3.2.1.133, genetically modified microorganism, Glucan 1,4-α-maltohydrolase, maltogenic α-amylase

## CONTENTS

# 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 microorganisms or products thereof including a product obtained by a fermentation process using microorganisms: (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 EU 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 Union list may be placed on the market as such and used I foods, in accordance with the specification and condition of use provided for in Article 7 (2) of Regulation (EC) No 1332/2008 on food enzymes.[3]

On 16 June 2022, a new application has been introduced by the applicant "DSM Food Specialties" for the authorization of the food enzyme Glucan 1,4-α-maltohydrolase from a genetically modified strain of *Bacillus subtilis* (strain MAM).

### 1.1.2 | Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessment and the assessment of possible confidentiality requests of the following food enzyme: Glucan 1,4-α-maltohydrolase from a genetically modified strain of *Bacillus subtilis* (strain MAM), in accordance with Regulation (EC) No 1331/2008 establishing a common authorization procedure for food additives, food enzymes and food flavourings.

### 1.1.3 | Interpretation of the Terms of Reference

During the risk assessment, the production strain was renamed *Bacillus subtilis* strain MAMDSM in order to be unambiguously identified.

# 2 | DATA AND METHODOLOGIES

## 2.1 | Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme glucan 1,4-α-maltohydrolase from a genetically modified *Bacillus subtilis* strain MAMDSM. The dossier was updated on 18 January 2023.

[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, 11.03.2011, pp. 15–24.

## 2.2 | Methodologies

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

The current 'Scientific Guidance for the submission of dossiers on Food Enzymes' (EFSA CEP Panel, 2021) has been followed for the evaluation of the application.

## 2.3 | Public consultation

According to Article 32c(2) of Regulation (EC) No 178/2002[4] and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations, EFSA carried out a public consultation on the non-confidential version of the technical dossier from 20 September to 11 October 2023 for which no comments were received.

# 3 | ASSESSMENT

| | |
|---|---|
| IUBMB nomenclature | Glucan 1,4-$\alpha$-maltohydrolase |
| Systematic name | 4-$\alpha$-D-glucan $\alpha$-maltohydrolase |
| Synonyms | Maltogenic amylase; maltogenic $\alpha$-amylase |
| IUBMB No | EC 3.2.1.133 |
| CAS No | 160611-47-2 |
| EINECS No | 630-523-5 |

Glucan-1,4-$\alpha$-maltohydrolases catalyse the hydrolysis of (1–4)-$\alpha$-D-glucosidic linkages in starch polysaccharides to successively remove maltose residues from the non-reducing ends of the chains. The enzyme under assessment is intended to be used in processing of cereals and other grains for the production of baked and brewed products.

## 3.1 | Source of the food enzyme[5]

The glucan-1,4-$\alpha$-maltohydrolase is produced with the genetically modified bacterium *Bacillus subtilis* strain MAMDSM (DS 79893), which is deposited at the Westerdijk Fungal Biodiversity Institute (the Netherlands) with the deposit number CBS 147475.[6] The production strain was identified as *B. subtilis* by whole genome sequence (WGS) and *de novo* assembly analysis of seven closely related genomes, with an average nucleotide identity (ANI) of 99.9% compared to the type strain *B. subtilis* 168 from which it derives.[7,8]

The species *B. subtilis* 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 genes and toxigenic activity are verified for the specific strain used (EFSA BIOHAZ Panel, 2022). A cytotoxicity test made with supernatants indicated that the production strain *B. subtilis* MAMDSM did not induce cell damage to CHO-K1 cells using the lactate dehydrogenase assay.[9] WGS analysis of the production strain did not identify antimicrobial resistance genes of concerns.[10]

### 3.1.1 | Characteristics of the parental and recipient microorganisms

The parental microorganism is *Bacillus subtilis* strain 168 (DS 20887). The recipient strain [redacted] was developed from the parental strain by classical mutagenesis and selection for the absence of sporulation [redacted] and of $\alpha$-amylase production [redacted]. This was followed by the disruption of the [redacted] and the [redacted].[7]

[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, p. 1–24.

[5]Technical Dossier/Risk Assessment/Source of the food enzyme/Source of the food enzyme.

[6]Technical Dossier/Risk Assessment/Source of the food enzyme/Annex 17.

[7]Technical Dossier/Risk Assessment/Source of the food enzyme/Annex 13.

[8]2023-06-20_Reply to ADR/ Source of the food enzyme/Annex 13.A.

[9]Technical Dossier/Risk Assessment/Source of the food enzyme/Annex 10.

[10]Technical Dossier/Risk Assessment/Source of the food enzyme/Annex 13 Appendix 4.

During the development of the recipient strain, plasmids were used containing genes conferring resistance to bleomycin, kanamycin and neomycin. The absence of backbone sequences from these vectors was confirmed by WGS analysis.[11]

### 3.1.2 | Characteristics of introduced sequences

The sequence encoding the glucan 1,4-$\alpha$-maltohydrolase [redacted] was obtained from [redacted].

The plasmid [redacted] contained the [redacted] gene placed between by the 5′ and 3′ flanking sequences of the *B. subtilis* [redacted] gene. The plasmid [redacted], used for the deletion of the [redacted] gene, carried the 5′ and 3′ flanking sequences of [redacted].[12] The two vectors were constructed from the [redacted] plasmids [redacted] and [redacted]. They carried the [redacted], an interrupted [redacted] gene [redacted] and a [redacted] gene [redacted], both from [redacted] and a [redacted] gene [redacted] from [redacted].[13]

### 3.1.3 | Description of the genetic modification

The purpose of the genetic modification was to enable the production strain to produce glucan-1,4-$\alpha$-maltohydrolase from [redacted]. For this purpose, the vectors [redacted] and [redacted] were inserted by [redacted] into the [redacted] and the [redacted] loci, respectively, of the recipient strain [redacted]. The vector backbones were then excised via [redacted] between [redacted] sequences, leading to the insertion of the [redacted] expression module into the [redacted] gene and to the excision of the originally [redacted] gene. The resulting strain was subjected to conventional mutagenesis, and the production strain was selected based on increased enzyme production.

WGS analysis of the production strain confirmed the integration of the [redacted] gene into the [redacted] locus and the deletion of the [redacted], [redacted] and [redacted] genes. The genes flanking the [redacted] locus ([redacted] and [redacted], encoding a [redacted] and a [redacted], respectively) were also partially deleted.[7]

### 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.

The production strain *B. subtilis* MAMDSM differs from the recipient strain DS 18174 in its capacity to overproduce the glucan-1,4-$\alpha$-maltohydrolase. The absence of the antibiotic resistance genes used during the genetic modifications, including those for the development of the recipient strain, was confirmed by WGS analysis.[7]

No issues of concern arising from the genetic modifications were identified by the Panel. As the other qualifications have been met, the production strain was considered to qualify for the QPS approach.

## 3.2 | Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[14], with food safety procedures based on Hazard Analysis and Critical Control Points and in accordance with current Good Manufacturing Practice.[15]

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. The filtrate containing the food enzyme is then 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.[16] 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.

[11]Technical Dossier/Risk Assessment/Source of the food enzyme/Annex 14.

[12]Technical Dossier/Risk Assessment/Source of the food enzyme/Annex 15.

[13]Technical Dossier/Risk Assessment/Source of the food enzyme/Source of the food enzyme pp. 7–8 and Annex 15.

[14]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.

[15]Technical Dossier/Risk Assessment/Manufacturing process/p. 1/Annex 7.

[16]Technical Dossier/Risk Assessment/Manufacturing process/pp. 1-9/Annex 8.

## 3.3 | Characteristics of the food enzyme

### 3.3.1 | Properties of the food enzyme

The mature glucan-1,4-$\alpha$-maltohydrolase is a single polypeptide chain of 686 amino acids.[17] The molecular mass of the mature protein, calculated from the amino acid sequence, is around 75 kDa.[18] The food enzyme was analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis.[19] A consistent protein pattern was observed across all batches. The gels showed a major protein band corresponding to an apparent molecular mass of about 66 kDa, consistent with the expected mass of the enzyme. No other enzyme activities were reported.[20]

The in-house determination of glucan-1,4-$\alpha$-maltohydrolase activity is based on the hydrolysis of maltotriose (reaction conditions: pH 5.0, 37°C), measuring the release of glucose with a commercial hexokinase test. The enzyme activity is expressed in new maltogenic amylase units (NMAU)/g. One NMAU is defined as the amount of enzyme that liberates 1 µmol glucose per minute under the conditions of the assay.[21]

The food enzyme has a temperature optimum between 60°C and 70°C (pH 5.0) and a pH optimum around 5.0 (37°C). Thermostability was tested after a pre-incubation of the food enzyme for 15 min at different temperatures (pH 5.0). The enzyme activity decreased above 75°C, showing no residual activity after pre-incubation at 85°C.[22]

### 3.3.2 | Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches intended for commercialisation (Table 1).[23] The mean total organic solids (TOS) of the three food enzyme batches was 5.3% and the mean enzyme activity/TOS ratio was 404 NMAU/mg TOS.

**TABLE 1** Composition of the food enzyme

| Parameters | Unit | Batches | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| **Glucan-1,4-α-maltohydrolase activity** | NMAU/g[a] | 16,400 | 16,300 | 30,500 |
| **Protein** | % | 2.8 | 3.1 | 5.9 |
| **Ash** | % | 0.7 | 0.3 | 0.7 |
| **Water** | % | 94.2 | 96.3 | 91.9 |
| **Total organic solids (TOS)**[b] | % | 5.1 | 3.4 | 7.4 |
| **Activity/TOS ratio** | NMAU/mg TOS | 322 | 479 | 412 |

[a]MANU: new maltogenic amylase (NMAU), Unit/g (see Section 3.3.1).

[b]TOS calculated as 100% – % water – % ash.

### 3.3.3 | Purity

The lead content in the three commercial batches was below 5 mg/kg[24,25] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).

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.[24]

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

[17]Technical Dossier/Risk Assessment/Chemical composition, properties and purity/p. 3/Annex 13.

[18]Technical Dossier/Risk Assessment/Chemical composition, properties and purity/p. 3.

[19]Technical Dossier/Risk Assessment/Chemical composition, properties and purity/p. 3/Annex 3.

[20]Technical Dossier/Risk Assessment/Chemical composition, properties and purity/p. 4.

[21]Technical Dossier/Risk Assessment/Chemical composition, properties and purity/p. 3/Annex 2.

[22]Technical Dossier/Risk Assessment/Chemical composition, properties and purity/pp. 4–5/Annex 6.

[23]Technical Dossier/Risk Assessment/Chemical composition, properties and purity/p. 1/Annex 4; Methods of analysis/Annex 1, Annex 2.

[24]Technical Dossier/Risk Assessment/Chemical composition, properties and purity/Annex 4, Annex 5.

[25]LoD: Pb = 0.001 mg/kg.

### 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 in three independent batches analysed in triplicate. One gram of product was mixed with 100 mL of non-selective agar medium and poured into plates that were incubated at 30°C for 2 days. No colonies were produced. A positive control was included.[26]

The absence of recombinant DNA in the food enzyme was demonstrated by polymerase chain reaction (PCR) analysis of three batches in triplicate. No DNA was detected with primers that would amplify a 420-bp fragment specific for *B. subtilis* strain MAMDSM, with a limit of detection of 10 ng spiked DNA/mL food enzyme.[27,28]

## 3.4 | Toxicological data

As the production strain qualifies for the QPS approach to safety assessment and no issue of concern arising from the production process of the food enzyme was 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, 2021).

### 3.4.1 | Allergenicity

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

The potential allergenicity of the glucan-1,4-α-maltohydrolase produced with the genetically modified *B. subtilis* strain MAMDSM 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 from *Aspergillus oryzae*; Asp f 13, a serine protease from *Aspergillus fumigatus*; Sch c 1, a glucoamylase from *Schizophyllum commune*; Aed a 4, an α-glucosidase from *Aedes aegypti* (yellow fever mosquito).[29]

No information was available on oral- or respiratory sensitisation and elicitation reactions to this glucan-1,4-α-maltohydrolase.

The α-amylase from *A. oryzae* (Brisman, 2002; Brisman & Belin, 1991; Quirce et al., 1992, 2002; Sander et al., 1998), the serine protease from *A. fumigatus* (Kurup et al., 2002) and the 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 (Armentia et al., 2009; Cullinan et al., 1997; Poulsen, 2004). 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 respiratorily sensitised to α-amylase (Baur & Czuppon, 1995; Kanny & Moneret-Vautrin, 1995; Losada et al., 1992; Moreno-Ancillo et al., 2004; Quirce et al., 1992). Such information has not been reported for glucoamylase and serine protease.

Yeast extract, a known source of allergens, is present in the media fed to the microorganism. 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 no potentially allergenic residues from this source are present in the food enzyme.

The Panel considered that the risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded, but the likelihood is low.

## 3.5 | Dietary exposure

### 3.5.1 | Intended use of the food enzyme

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

[26]Technical Dossier/Risk Assessment/Manufacturing process/p. 1/Annex 18.

[27]Technical Dossier/Risk Assessment/Source of the food enzyme/Annex 16.

[28]2023-06-20_Reply to ADR/ Source of the food enzyme/Annex 16.

[29]Technical Dossier/Risk Assessment/Allergenicity/Annex 11.

**TABLE 2** Intended uses and recommended use levels of the food enzyme as provided by the applicant[c]

| Food manufacturing process[a] | Raw material (RM) | Recommended use level (mg TOS/kg RM)[b] |
|---|---|---|
| Processing of cereals and other grains | | |
| Production of baked products | Flour | 0.25–**6.8** |
| Production of brewed products | Cereals | 5.0–**39.6** |

Abbreviation: TOS, total organic solids.

[a]The name has been harmonised by EFSA according to the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023).

[b]The numbers in bold were used for calculation.

[c]Technical dossier/proposed normal and maximum use levels, p. 1.

In the baking processes, the food enzyme is added to flour during the preparation of the dough.[30] The conversion of starch lowers the rate of starch retrogradation, thereby reducing staling, and improves crumb structure.[31] The food enzyme–TOS remains in the final baked foods.

In the brewing processes, the food enzyme is added during the mashing step[32] where it hydrolyses the starch in the mash into fermentable sugars, i.e. maltose. The more uniform formation of fermentable sugars improves yield and consistency of the products.[33] The food enzyme–TOS remains in the final foods.

Based on data provided on thermostability (see Section 3.3.1) and the downstream processing step applied in the food processes, it is expected that the food enzyme is inactivated during the production of baked and brewed products.

### 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, 2021). The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP Panel, 2023). 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 1 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 48 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 26 European countries (Appendix B). The highest dietary exposure was estimated to be about 0.204 mg TOS/kg bw per day in adults at the 95th percentile.

**TABLE 3** Summary of the estimated dietary exposure to food enzyme–TOS in six population groups

| | Estimated exposure (mg TOS/kg body weight per day) | | | | | |
|---|---|---|---|---|---|---|
| **Population group** | **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) | 0.001–0.019 (12) | 0.015–0.041 (15) | 0.017–0.039 (19) | 0.011–0.028 (21) | 0.011–0.056 (22) | 0.010–0.031 (23) |
| **Min–max 95th percentile** (number of surveys) | 0.007–0.081 (11) | 0.036–0.069 (14) | 0.032–0.074 (19) | 0.020–0.056 (20) | 0.033–0.204 (22) | 0.025–0.098 (22) |

Abbreviation: TOS, total organic solids.

[30]Technical dossier/intended use(s) in food, p. 1.

[31]Technical dossier/THE TECHNOLOGICAL NEED, INCLUDING A DESCRIPTION OF THE TYPICAL PROCESSES IN WHICH THE ENZYME MAY BE APPLIED, p. 2.

[32]Technical dossier/intended use(s) in food, p. 2.

[33]Technical dossier/intended use(s) in food, p. 3.

### 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.

**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** | |
| Exposure to food enzyme–TOS always calculated based on the recommended maximum use level | + |
| Selection of broad FoodEx categories for the exposure assessment | + |
| Use of recipe fractions to disaggregate FoodEx categories | +/– |
| Use of technical factors in the exposure model | +/– |

Abbreviations: +, uncertainty with potential to cause overestimation of exposure; –, uncertainty with potential to cause underestimation of exposure.

The conservative approach applied to estimate the exposure to the 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

Since no toxicological assessment was considered necessary by the Panel, the margin of exposure was not calculated.

# 4 | CONCLUSIONS

Based on the data provided, the Panel concluded that the food enzyme glucan 1,4-$\alpha$-maltohydrolase produced with the genetically modified *Bacillus subtilis* strain MAMDSM 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

Glucan 1,4-$\alpha$-maltohydrolase from *Bacillus subtilis* strain MAM. January 2023. Submitted by DSM Food Specialties.

Additional information. 20 June 2023. Submitted by DSM Food Specialties.

## ABBREVIATIONS

| | |
|---|---|
| bw | body weight |
| CAS | Chemical Abstracts Service |
| CEP | EFSA Panel on Food Contact Materials, Enzymes and Processing Aids |
| EINECS | European Inventory of Existing Commercial Chemical Substances |
| FAO | Food and Agricultural Organization of the United Nations |
| GMM | genetically modified microorganism |
| IUBMB | International Union of Biochemistry and Molecular Biology |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| kDa | Kilodalton |
| LOD | limit of detection |
| PCR | polymerase chain reaction |
| QPS | qualified presumption of safety |
| SDS-PAGE | sodium dodecyl sulphate-polyacrylamide gel electrophoresis |
| TOS | total organic solids |
| WGS | whole genome sequencing |
| WHO | World Health Organization |

## CONFLICT 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.

## REQUESTOR

European Commission

## QUESTION NUMBER

EFSA-Q-2022-00603

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## LEGAL NOTICE



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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré, C., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Roos, Y., Andryszkiewicz, M., … Chesson, A. (2023). Safety evaluation of the food enzyme glucan 1,4-$\alpha$-maltohydrolase from the genetically modified *Bacillus subtilis* strain MAMDSM. *EFSA Journal*, *21*(11), e8410. https://doi.org/10.2903/j.efsa.2023.8410

## APPENDIX A

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

Appendix A can be found in the online version of this output (in the 'Supporting 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 1 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, Spain |
| **Toddlers** | From 12 months up to and including 35 months of age | Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, the Netherlands, Portugal, Republic of North Macedonia[b], Serbia[b], 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, the Netherlands, Portugal, Republic of North Macedonia[b], Serbia[b], Spain, Sweden |
| **Adolescents** | From 10 years up to and including 17 years of age | Austria, Belgium, Bosnia and Herzegovina[b], Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Montenegro[b], the Netherlands, Portugal, Romania, Serbia[b], Slovenia, Spain, Sweden |
| **Adults** | From 18 years up to and including 64 years of age | Austria, Belgium, Bosnia and Herzegovin[b], Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro[b], the Netherlands, Portugal, Romania, Serbia[b], 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, Montenegro[b], the Netherlands, Portugal, Romania, Serbia[b], 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).

[b] Consumption data from these pre-accession countries are not reported in Table 3 of this opinion, however, they are included in Appendix B for testing purpose.