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SCIENTIFIC OPINION

Safety evaluation of the food enzyme non-reducing end α-L-arabinofuranosidase from the non-genetically modified *Aspergillus tubingensis* strain ARF

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts

Abstract

The food enzyme non-reducing end α-L-arabinofuranosidase (α-L-arabinofuranoside non-reducing end-α-L-arabinofuranosidase; EC 3.2.1.55) is produced with the non-genetically modified *Aspergillus tubingensis* strain ARF by DSM Food Specialties B.V. The food enzyme was free from viable cells of the production organism. The food enzyme is intended to be used in five food manufacturing processes. Dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.455 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not indicate a safety concern. The systemic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rats. The Panel identified a no observed adverse effect level of 234 mg TOS/kg bw per day, the highest dose tested, which when compared with the estimated dietary exposure, results in a margin of exposure of at least 514. A search for the homology of the amino acid sequences of the non-reducing end α-L-arabinofuranosidase to known allergens was made and no match was found. The Panel considered that a risk of allergic reactions upon dietary exposure to the food enzyme 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

Aspergillus tubingensis, EC 3.2.1.55, EFSA-Q-2014-00671, food enzyme, non-reducing end α-L-arabinofuranosidase, α-L-arabinofuranosidase

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 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.

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 "Genecor International B.V.", "Amano Enzyme Inc" and "DSM Food Specialties B.V." for the authorization of 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 Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008, the Commission has verified that the three applications fall within the scope of the food enzyme Regulation and contain all the elements required under Chapter II of that Regulation.

1.1.2 | Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes 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 on food enzymes.

[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.

1.2 | Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of the food enzyme arabinofuranosidase from *Aspergillus niger* (strain ARF).

Recent data identified the production microorganism as *Aspergillus tubingensis* (Section 3.1). Therefore, this name will be used in this opinion instead of *Aspergillus niger*.

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme arabinofuranosidase from *Aspergillus niger* (strain ARF).

Additional information was requested from the applicant during the assessment process on 7 September 2022 and received on 27 September 2023 (see 'Documentation provided to EFSA').

Following the request for additional data sent by EFSA on 7 September 2022, a clarification teleconference was held on 24 October 2022, after which the applicant spontaneously provided additional data on 15 November 2022.

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 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA, 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. Additional information was requested in accordance with the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021) and the guidance on the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023).

3 | ASSESSMENT

IUBMB nomenclature	Non-reducing end α-L-arabinofuranosidase
Systematic name	α-L-Arabinofuranoside non-reducing end α-L-arabinofuranosidase
Synonyms	α-Arabinofuranosidase; α-arabinosidase
IUBMB No	EC 3.2.1.55
CAS No	9067-74-7
EINECS No	232-957-7

Non-reducing end α-L-arabinofuranosidases catalyse the hydrolytic release of L-arabinofuranoside residues from the non-reducing ends of α-L-arabinans and arabinoxylans.

The food enzyme under assessment is intended to be used in five food manufacturing processes as described in the EFSA guidance (EFSA CEP Panel, 2023): processing of cereals and other grains for the production of (1) baked products and (2) cereal-based products other than baked; processing of fruits and vegetables for the production of (3) juices, (4) fruit and vegetable products other than juices and (5) wine and wine vinegar.

3.1 | Source of the food enzyme

The α-L-arabinofuranosidase is produced with the filamentous fungus *Aspergillus tubingensis* strain [redacted], which is deposited as *Aspergillus niger* at the [redacted] with deposition number [redacted].[4] The production strain was identified as *A. tubingensis* by [redacted][5]

A. tubingensis ARF was obtained by [redacted].[6]

[4]Technical dossier/Additional information September 2023/Annex I.

[5]Technical dossier/Additional information September 2023/Annex IV.

[6]Technical dossier/Additional information September 2023.

3.2 | Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004,[7] with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[8]

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 enzyme is further purified and concentrated, including an ultrafiltration step in which the enzyme protein is retained, while most of the low molecular mass material passes the filtration membrane and is discarded.[9] 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.[10]

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 non-reducing end α-L-arabinofuranosidase has [redacted] which are single polypeptide chains of [redacted] amino acids, respectively.[11] The molecular masses of the mature proteins of the forms [redacted], calculated from the amino acid sequences, are [redacted], respectively.[12] The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. A consistent protein pattern was observed across all batches, showing major protein bands migrating at about [redacted].[13]

The food enzyme was tested for endo 1,5-α-arabinanase, pectin lyase and endo-1,4-β-xylanase activities, and all were detected. No other enzyme activities were reported.[14]

The applicant's in-house determination of non-reducing end α-L-arabinofuranosidase activity is based on the hydrolysis of *p*-nitrophenyl-α-L-arabinofuranoside (reaction conditions: pH 4.4, 37°C). The enzymatic activity is determined by measuring the release of *p*-nitrophenol spectrophotometrically at 405 nm. The enzyme activity is expressed in ARF Units (ARF)/g. One ARF is defined as the amount of enzyme that releases 1 nmol *p*-nitrophenol from *p*-nitrophenyl-α-L-arabinofuranoside per second under the conditions of the assay.[15]

The food enzyme has a temperature optimum around 65°C (pH 4.4) and a pH optimum between pH 3.5 and 4.5 (37°C). Thermostability was tested after pre-incubation of the food enzyme at different temperatures and for different time periods. Enzyme activity decreased above 50°C showing no residual activity at 70°C after 60 min of pre-incubation, and at 75°C after 2 min.[16]

3.3.2 | Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation, one of which was used for toxicological testing, and for one batch produced for additional toxicological tests (Table 1).[17] The mean total organic solids (TOS) of the three food enzyme batches used for commercialisation is 22.2% and the mean enzyme activity/TOS ratio is 186.7 ARF/mg TOS.

[7]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.
[8]Technical dossier/pg. 11, 53/Annex 5.
[9]Technical dossier/pg. 53–60/Annex 6.
[10]Technical dossier/pg. 5/Annex 7.
[11]Technical dossier/pg. 10, 43–44 and additional information December 2023 (Part II)/Annex IV/Appendix 3.
[12]Technical dossier/pg. 10, 43–44 and additional information December 2023 (Part II)/Answer 6.
[13]Technical dossier/pg. 10, 40–41 and additional information December 2023 (Part I)/Answer 7.
[14]Technical dossier/pg. 9–10, 45.
[15]Technical dossier/pg. 10, 44–45/Annex 2.
[16]Technical dossier/pg. 10, 45–46 and additional information December 2023 (Part I)/Answer 8.
[17]Technical dossier/pg. 39–40, 75/Annexes: 1, 2 and 3.

TABLE 1 Composition of the food enzyme.

Parameters	Unit	Batches			
		1	2	3[a]	4[b]
Non-reducing end α-L-arabinofuranosidase activity	ARF/g[c]	53,750	30,100	41,600	27,050
Protein	%	20.2	11.3	13.6	10.8
Ash	%	0.9	0.8	0.6	0.9
Water	%	72.5	82.5	76.0	80.8
Total organic solids (TOS)[d]	%	26.6	16.7	23.4	18.3
Activity/TOS ratio	ARF/mg TOS	202.1	180.2	177.8	147.8

Abbreviation: TOS, total organic solids.

[a]Batch used for the Ames test, in vitro mammalian chromosomal aberration test and the repeated dose 90-day oral toxicity study.

[b]Batch used for the in vitro mammalian cell micronucleus test.

[c]ARF Arabinofuranosidase Unit (see Section 3.3.1).

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

3.3.3 | Purity

The lead content in the three commercial batches and in the batch used for toxicological studies was below 5 mg/kg[18,19] 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).[20] No antimicrobial activity was detected in any of the tested batches.[21]

Strains of *Aspergillus*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Frisvad et al., 2018). The presence of aflatoxins, fumonisins, ochratoxin A, zearalenone and trichothecenes was examined in all food enzyme batches and was below the limit of detection (LoD) of the applied methods.[22,23] Adverse effects caused by the potential presence of other secondary metabolites are addressed by the toxicological examination of the food enzyme–TOS.

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

3.3.4 | Viable cells 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. [redacted]. A positive control was included.[24]

3.4 | Toxicological data

A battery of toxicological tests including a bacterial reverse mutation test (Ames test), an in vitro mammalian chromosomal aberration test, an in vitro mammalian cell micronucleus test and a repeated dose 90-day oral toxicity study in rats has been provided.

Batch 3 is a commercial batch and batch 4 (Table 1) has a similar protein pattern and composition as the batches used for commercialisation. Hence, both are considered suitable as test items.

[18]Technical dossier/pg. 10, 43/Annexes: 3 and 4 and additional information December 2023 (Part I)/Answer 9.

[19]LoDs: Pb = 2, 1 and 0.6 mg/kg.

[20]Technical dossier/pg. 10, 43/Annexes: 3 and 4.

[21]Technical dossier/pg. 10, 43/Annexes: 3 and 4.

[22]Technical dossier/pg. 10, 43/Annexes: 3 and 4.

[23]LoDs: aflatoxins and ochratoxin A = 0.1 μg/kg each; fumonisins and trichothecenes = 10 μg/kg each; zearalenone = 3 μg/kg.

[24]Technical dossier/Additional information September 2023/Annex V.

3.4.1 | Genotoxicity

3.4.1.1 | *Bacterial reverse mutation test*

A bacterial reverse mutation test (Ames test) was performed according to the Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997a) and following Good Laboratory Practice (GLP).[25] Four strains of Salmonella Typhimurium (TA98, TA100, TA1535 and TA1537) and *E. coli* WP2*uvrA* (pKM101) were used with or without metabolic activation (S9-mix), applying the pre-incubation method.

Two experiments were carried out in triplicate, using five concentrations of the food enzyme ranging from 50 to 5000 µg dry matter/plate, corresponding to 49 to 4875 µg TOS/plate in the first experiment and from 100 to 5000 µg dry matter/plate, corresponding to 98 to 4875 µg TOS/plate in the second experiment.

No cytotoxicity was observed at any concentration of the test substance.

Upon treatment with the food enzyme, there was no biologically relevant increase in the number of revertant colonies above the control values, in any strain tested, with or without S9-mix.

The Panel concluded that the food enzyme non-reducing end α-L-arabinofuranosidase did not induce gene mutations under the test conditions applied in this study.

3.4.1.2 | In vitro *mammalian chromosomal aberration test*

The in vitro mammalian chromosomal aberration test was carried out according to OECD Test Guideline 473 (OECD, 1997b) and following GLP.[26] Two separate experiments were performed with quintuplicate cultures of a Chinese Hamster Ovary cell line. The cell cultures were treated with the food enzyme either with or without metabolic activation (S9-mix).

In a range-finding test, no cytotoxicity above 50% was seen at any concentration tested up to 5000 µg dry matter/mL, corresponding to 4875 µg TOS/mL plate. In the first experiment, cells were exposed to the food enzyme and scored for chromosomal aberrations at concentrations of 1250, 2500 and 5000 µg dry matter/mL, corresponding to 1219, 2438 and 4875 µg TOS/mL in a short-term treatment (3 h-exposure and 18 h-recovery period) either with or without S9-mix. In the second experiment, cells were exposed to the food enzyme and scored for chromosomal aberrations at concentrations of 1000, 2250 and 5000 µg dry matter/mL, corresponding to 975, 2194 and 4875 µg TOS/mL in a long-term treatment (19.5 h-continuous exposure and 1.5 h-recovery period) without S9-mix.

No cytotoxicity was seen either in the short-term treatment (with or without S9-mix) or in long-term treatment. The frequency of structural chromosomal aberrations was not statistically significantly different to the negative controls at all concentrations tested. The numerical chromosomal aberrations were not reported.

The Panel concluded that the food enzyme non-reducing end α-L-arabinofuranosidase did not induce an increase in the frequency of structural chromosomal aberrations under the test conditions applied in this study.

3.4.1.3 | In vitro *mammalian cell micronucleus test*

The in vitro mammalian cell micronucleus test was carried out according to OECD Test Guideline 487 (OECD, 2016) and following GLP.[27] Two separate experiments were performed with duplicate cultures of human peripheral whole blood lymphocytes. The cell cultures were treated with the food enzyme with or without metabolic activation (S9-mix).

In the first experiment, cells were exposed to the food enzyme and scored for the frequency of bi-nucleated cells with micronuclei (MNBN) at concentrations of 950, 1900 and 4750 µg TOS/mL in a short-term treatment (4 h-exposure and 40 h-recovery period) either with or without S9-mix.

In the second experiment, cells were exposed to the food enzyme and scored for MNBN at concentrations of 950, 1900 and 2850 µg TOS/mL in a long-term treatment (44 h-continuous exposure) without S9-mix.

No cytotoxicity was seen in the short-term treatment with or without S9-mix. In the long-term treatment, cytotoxicity of 56% (based on relative cell growth) was observed at the highest concentration tested.

The frequency of MNBN was statistically significantly different to the negative controls at concentration of 900 µg TOS/mL tested without S9-mix in the short-term treatment. However, the reported value was within the 95% of the historical control range and no concentration response was observed. The frequency of MNBN was not statistically significantly different to the negative controls at all other concentrations tested.

The Panel concluded that the food enzyme non-reducing end α-L-arabinofuranosidase did not induce an increase in the frequency of MNBNs under the test conditions applied in this study.

[25]Technical report p. 16/Annex 13.

[26]Technical report p. 16/Annex 14.

[27]Technical dossier/Additional information December 2023/Answer 10 and Annex II.

3.4.2 | Repeated dose 90-day oral toxicity study in rodents

The repeated dose 90-day oral toxicity study was performed in accordance with OECD Test Guideline 408 (OECD, 1998) and following GLP.[28]

Groups of 10 male and 10 female Wistar rats (HsdCpb:WU) received the food enzyme by gavage in doses of 50, 250 or 1000 mg/kg body weight (bw) per day, corresponding to 12, 59 or 234 mg TOS/kg bw per day. Controls received the vehicle (double distilled water).

No mortality was observed.

The body weight gain was statistically significantly increased at week one and four of administration in low-dose females (+16% and +11%). The Panel considered the changes as not toxicologically relevant as they were only recorded sporadically, as they were only observed in one sex, there was no dose–response relationship and the changes were without a statistically significant effect on the final body weight gain.

The feed consumption was statistically significantly increased at week three of administration in mid-dose males (+5%). The Panel considered the change as not toxicologically relevant as it was only recorded sporadically, as it was only observed in one sex, there was no dose–response relationship, there was no statistically significant change in the final feed consumption and there were no statistically significant changes in the final body weight and body weight gain.

In the functional observations, a statistically significant decrease in the grip strength in hind limbs was observed in high-dose males (−6%). The Panel considered the change as not toxicologically relevant as it was only observed in one sex and the change was small.

Haematological investigations revealed a statistically significant increase in the haematocrit in low-dose males (+6%), in the mean corpuscular haemoglobin (MCH) in mid- and high-dose females (both +4%) and in the mean corpuscular haemoglobin concentration (MCHC) in high-dose females (+3%). The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (all parameters), there was no dose–response relationship (haematocrit and MCH), the changes were small (all parameters) and there were no changes in other relevant parameters (red blood cell count).

Clinical chemistry investigations revealed a statistically significant increase in sodium in all treated male groups (+1%, +3% and +2%), in calcium in mid- and high-dose males (+3% and +4%), in chloride in mid- and high-dose males (both +2%), in blood urea nitrogen (BUN) in low-dose males (+10%), in urea in low-dose males (+9%), in inorganic phosphorus in low-dose males (+23%) and in mid- and high-dose females (+22% and +23%), in total bilirubin (TB) in low-dose females (+3%) and a decrease in chloride in low-dose females (−2%). The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (sodium, calcium, BUN, urea and TB), there was no consistency between the changes in males and females (chloride), there was no clear dose–response relationship (all parameters) and the changes were small (sodium, calcium, chloride and TB).

No other statistically significant or biologically relevant differences from controls were reported.

The Panel identified a no observed adverse effect level (NOAEL) of 234 mg TOS/kg bw per day, the highest dose tested.

3.4.3 | Allergenicity

The allergenicity assessment considers only the food enzyme and not additives, carriers or other excipients, which may be used in the final formulation.

The potential allergenicity of the non-reducing end α-L-arabinofuranosidase produced with the *A. tubingensis* strain ARF was assessed by comparing its amino acid sequences with those of known allergens as described in the EFSA GMO Scientific Opinion (EFSA GMO Panel, 2010). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, no match was found in the AllergenOnline database.[29]

No reports on oral or respiratory sensitisation or elicitation reactions of the non-reducing end α-L-arabinofuranosidase under assessment have been published.[30]

Respiratory allergy following inhalation of pollen from *Prosopis velutina* has been reported, and IgE binding to α-L-arabinofuranosidase was shown (Huerta-Ocampo et al., 2020). However, several studies have shown that individuals respiratorily sensitised to a food enzyme are usually able to ingest the corresponding enzyme without acquiring clinical symptoms of food allergy (Armentia et al., 2009; Cullinan et al., 1997; Poulsen, 2004). Adverse reactions upon dietary exposure to α-L-arabinofuranosidases in individuals sensitised through the respiratory route have not been reported.

The Panel considered that the results of the sequence homology search and the available literature do not indicate a risk of allergic reactions upon dietary exposure to the non-reducing end α-L-arabinofuranosidase under assessment.

The production strain belongs to the *Aspergillus* genus, which is known to cause respiratory allergy (Kurup et al., 2000; Shen & Han, 1998; Vermani et al., 2015). Allergic reactions upon dietary exposure have been observed, but are rare (Xing et al., 2022). The biomass is removed during the production process; however, allergenic proteins of the production strain can be released into the culture medium from which the food enzyme is obtained.

[28]Technical dossier/Annex 15.

[29]Technical dossier/p. 14, 76–78/Annexes: 16 and 17 and additional information December 2023/Answer 6 and Annex VI.

[30]Technical dossier/Additional information December 2023/Answer 11 and Annex III.

Cereal bran, a product from cereals that may cause allergies or intolerances (listed in the Regulation (EU) No 1169/2011[31]), is used as raw material. In addition, brewer's yeast, a known source of allergens, is also present in the medium fed to the production strain. During the fermentation process, these products will mostly be degraded and utilised by the production strain.

Taken together, concerning the potential allergic reactions due to the production strain and the raw material in the culture medium of the microorganism, the Panel considered that residual amounts of allergenic proteins could be present in the food enzyme. Taking into account the level of dietary exposure (see Section 3.5.2), this would result in minute amounts in the final foods, from which allergic reactions are usually not expected.

In conclusion, the Panel considered that under the conditions of use, a risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded, but that 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 five 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.[32]

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	0.1–**2.5**
• Production of cereal-based products other than baked[33]	Flour	0.1–**2.5**
Processing of fruits and vegetables		
• Production of juices	Fruit and vegetables	1–**10**
• Production of fruit and vegetable products other than juices[34]	Fruit and vegetables	1–**10**
• Production of wine and wine vinegar	Grapes	1–**10**

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

[b]The numbers in bold represent the maximum recommended use levels, which were used for calculation.

In the production of baked and other cereal-based products, the food enzyme is added to flour during dough making.[35] The food enzyme hydrolyses hemicelluloses, improving dough handling and reducing batter viscosity.[36] The food enzyme–TOS remain in the final foods.

In the production of juices, the food enzyme is added to fruit or vegetables during crushing or to the pressed juices before depectinisation.[37] The enzymatic treatment decreases viscosity and improves the yield.[38] The food enzyme–TOS remain in the juices.

In the production of fruit and vegetable products other than juices, the food enzyme is added to crushed fruits and vegetables.[39] The hydrolysis catalysed by the food enzyme contributes to cell wall degradation.[40] The food enzyme–TOS remain in the final fruit and vegetable products.

In the production of wine and wine vinegar, the food enzyme is added to grapes during crushing and maceration.[41] The enzymatic treatment increases the release of must, eases pressing and increases the yield.[42] The food enzyme can also be added after the fermentation[43] to clarify the wine. The food enzyme–TOS remain in the wine.

[31]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.

[32]Spontaneous information November 2022/07.2 Use levels _ ARF_ FV other than juices, and Additional information September 2023/Answer 12.

[33]Additional information September 2023/Answer 12.

[34]Spontaneous information November 2022/07.1 Intended Use.

[35]Technical dossier/p. 63.

[36]Technical dossier/p. 92.

[37]Technical dossier/p. 64.

[38]Technical dossier/p. 93.

[39]Spontaneous information November 2022/07.1 Intended Use/p. 2.

[40]Spontaneous information November 2022/12. RM - Intended use and Technological need_ARF_FV other than juices/p. 1.

[41]Technical dossier/p. 65.

[42]Technical dossier/p. 93.

[43]Technical dossier/p. 65.

Based on data provided on thermostability (see Section 3.3.1), the Panel considered that the food enzyme may remain in its active form in the food manufacturing processes listed in Table 2, depending on the processing conditions.

3.5.2 | Dietary exposure estimation

Chronic exposure to the food enzyme–TOS was calculated using the FEIM webtool[44] 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 0.455 mg TOS/kg bw per day in children at the 95th percentile.

TABLE 3 Summary of 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)	0.013–0.132 (11)	0.052–0.292 (15)	0.025–0.159 (19)	0.017–0.097 (21)	0.017–0.069 (22)	0.013–0.050 (22)
Min–max 95th (number of surveys)	0.044–0.287 (9)	0.166–0.437 (13)	0.059–0.455 (19)	0.042–0.272 (20)	0.054–0.218 (22)	0.050–0.159 (21)

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	
Selection of broad FoodEx categories for the exposure assessment	+
Exposure to food enzyme–TOS always calculated based on the recommended maximum use level	+
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; TOS, total organic solids.

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 an overestimation of the exposure.

[44]Version 1.1.1-1.

3.6 | Margin of exposure

A comparison of the NOAEL (234 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates of 0.013–0.292 mg TOS/kg bw per day at the mean and from 0.042 to 0.455 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposure of at least 514.

4 | CONCLUSIONS

Based on the data provided, the absence of issues of concern arising from the production process and the derived margin of exposure, the Panel concluded that the food enzyme non-reducing end α-L-arabinofuranosidase produced with the non-genetically modified *A. tubingensis* strain ARF does not give rise to safety concerns under the intended conditions of use.

5 | REMARK

The use of this non-reducing end α-L-arabinofuranosidase from the non-genetically modified *Aspergillus tubigensis* strain ARF is not considered to raise a safety concern when used in the production of fruit and vegetable juices. However, the Panel noted that according to the Directive 2012/12/EU, the use of non-reducing end α-L-arabinofuranosidase is not permitted in the treatment of fruits for juice production.

6 | DOCUMENTATION AS PROVIDED TO EFSA

α-L-arabinofuranosidase from *Aspergillus niger*. August 2014. Submitted by DSM Food Specialties B.V.
Additional information. September 2023. Submitted by DSM Food Specialties B.V.
Spontaneous information. November 2022. Submitted by DSM Food Specialties B.V.
Summary report on technical data and dietary exposure. April 2015. Delivered by Hylobates Consulting and BiCT, Italy.
Summary report on genotoxicity and subchronic toxicity study and allergenicity. FoBiG GmbH, Germany. 2015.

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
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
kDa	kiloDalton
LoD	bi-nucleated cells with micronuclei
MNBN	limit of detection
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
TOS	total organic solids
WHO	World Health Organization

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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.

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SUPPORTING INFORMATION

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

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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*, Serbia*, 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*, Serbia*, Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Bosnia and Herzegovina*, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Montenegro*, the Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Bosnia and Herzegovina*, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro*, the Netherlands, Portugal, Romania, Serbia*, 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*, the Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden

*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.

[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).