SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 19 May 2022

doi: 10.2903/j.efsa.2022.7375

Safety evaluation of the food enzyme cellulase from the genetically modified *Trichoderma reesei* strain AR-852

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Abstract

The food enzyme cellulase (4-(1,3;1,4)-β-D-glucan 4-glucanohydrolase; EC 3.2.1.4) is produced with the genetically modified *Trichoderma reesei* strain AR-852 by AB Enzymes GmbH. The genetic modifications did not give rise to safety concerns. The food enzyme is considered free from viable cells of the production organism and its DNA. The food enzyme is intended to be used in five food manufacturing processes: baking processes, brewing processes, distilled alcohol production, wine and wine vinegar production, and fruit and vegetable processing for juice production. As residual amounts of total organic solids (TOS) are removed by distillation, dietary exposure was only calculated for the other four food processes. Dietary exposure to the food enzyme TOS was estimated to be up to 0.1 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 1,000 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 10,000. A search for similarity of the amino acid sequence of the food enzyme to known allergens was made and no match was found. The Panel considered that, under the intended conditions of use (other than distilled alcohol production) the risk of allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but the likelihood for this to occur is considered to be 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: cellulase, 4-(1,3;1,4)-β-D-glucan 4-glucanohydrolase, EC 3.2.1.4, β-1,4-glucanase, *Trichoderma reesei*, genetically modified microorganism

Requestor: European Commission

Question number: EFSA-Q-2021-00306

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

Declarations of interest: The declarations of interest of all scientific experts active in EFSA's work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

Acknowledgements: The Panel wishes to thank the following for the support provided to this scientific output: Christine Horn. The Panel wishes to acknowledge all European competent institutions, Member State bodies and other organisations that provided data for this scientific output.

Suggested citation: EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré C, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mengelers M, Mortensen A, Rivière G, Steffensen I-L, Tlustos C, Van Loveren H, Vernis L, Zorn H, Glandorf B, Herman L, Aguilera J, Andryszkiewicz M, Liu Y, Nielsen E, Norby K, di Piazza G and Chesson A, 2022. Scientific Opinion on the safety evaluation of the food enzyme cellulase from the genetically modified *Trichoderma reesei* strain AR-852. EFSA Journal 2022;20(7):7375, 16 pp. https://doi.org/10.2903/j.efsa.2022.7375

ISSN: 1831-4732

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

† Deceased.

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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 "AB Enzymes GmbH" for the authorisation of the food enzyme Cellulase from a genetically modified strain of *Trichoderma reesei* (strain AR-852).

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 contains 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, the European Commission requests the European Food Safety Authority to carry out the safety assessment on the following food enzyme: Cellulase from a genetically modified strain of *Trichoderma reesei* (strain AR-852), in accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings.

[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.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme cellulase from a genetically modified strain of *Trichoderma reesei* (strain AR-852).

Additional information was requested from the applicant during the assessment process on 16 November 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) and following the relevant existing guidance of EFSA Scientific Committee.

The Guidance on the submission of a dossier on food enzymes for safety evaluation (EFSA CEF Panel, 2009a) as well as the Statement on characterisation of microorganisms used for the production of food enzymes (EFSA CEP Panel, 2019) have been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance to the updated Scientific Guidance for the submission of dossiers on food enzymes (EFSA CEP Panel, 2021a).

3. Assessment

IUBMB nomenclature	Cellulase
Systematic name	4-(1,3;1,4)-β-D-glucan 4-glucanohydrolase
Synonyms	β-1,4-glucanase; carboxymethyl cellulase
IUBMB No	EC 3.2.1.4
CAS No	9,012-54-8
EINECS No	232–734-4

Cellulase catalyses the random hydrolysis of 1–4-β-glycosidic linkages in cellulose and other β-glucans resulting in the generation of shorter β-D-glucan chains. The enzyme is intended to be used in baking processes, brewing processes, distilled alcohol production, wine production and fruit and vegetable processing for juice production.

3.1. Source of the food enzyme

The cellulase is produced with the genetically modified filamentous fungus *Trichoderma reesei* strain AR-852 ([redacted]), which is deposited at the Westerdijk Fungal Biodiversity Institute Culture Collection (CBS, the Netherlands), with deposit number [redacted][4] The production strain was identified as *T. reesei* [redacted][5]

3.1.1. Characteristics of the parental and recipient microorganisms

Strain [redacted] is [redacted] derived [redacted] from the parental strain QM6a (ATCC 13631). Taxonomic identification of *T. reesei* QM6a was performed [redacted] (Kuhls et al., 1996). [redacted][6]

[4] Technical dossier/Volume I/Annex 7 and Volume II/Annex 5.
[5] Technical dossier/Volume II/Annex 4.
[6] Technical Dossier/Volume II/Annex 1.

[redacted][8]

3.1.2. Characteristics of introduced sequences

[redacted][9]

3.1.3. Description of the genetic modification process

[redacted][10]

[redacted][12]

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]

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[13], with food safety procedures based on hazard analysis and critical control points, and in accordance with current good manufacturing practice.[14]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, batch fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration, leaving a supernatant containing the food enzyme. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in which enzyme protein is retained, while most of the low molecular mass material passes the filtration membrane and is discarded.[15] The applicant

[7] Technical Dossier/Volume II/Annex 2.
[8] Technical Dossier/Volume II/Annex 3.
[9] Technical Dossier/Volume II/Annex 10.
[10] Technical Dossier/Volume II/Annex 7.
[11] Technical Dossier/Volume II/Annex 14.
[12] Technical Dossier/Volume II/Annex 6.
[13] 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.
[14] Technical Dossier/Volume I/Annex 8.
[15] Technical Dossier/Volume I/pp.18-26/Annex 10.

provided information on the identity of the substances used to control the fermentation and in the subsequent downstream processing of the food enzyme.[16]

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 cellulase is a single polypeptide chain of [redacted] amino acids.[17] The molecular mass of the mature protein, calculated from the amino acid sequence, was [redacted] kDa.[18] The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). A consistent protein pattern was observed across all batches. The gel showed a single major protein band corresponding to an apparent molecular mass of about [redacted] kDa, consistent with the expected mass of the enzyme.[19] The food enzyme was tested for the presence of amylase and protease activities and neither were detected.[20] No other enzymatic activities were reported.

The in-house determination of cellulase activity is based on hydrolysis of a carboxymethyl cellulose solution (reaction conditions: pH 4.5, 30°C, 11 min). The enzymatic activity is determined by measuring the change of viscosity of the substrate. The cellulase activity is quantified relative to an internal enzyme standard and expressed in cellulase unit/mg (CU/mg). One cellulase unit (CU) is defined as the amount of activity resulting in a relative fluidity change of 1 in 5 min in a defined carboxymethyl cellulose solution under the conditions of the assay.[21]

The food enzyme has a temperature optimum around 55°C (pH 4.5) and a pH optimum around pH 4.5 (30°C). Thermostability was tested after a pre-incubation of the food enzyme at 85°C for different time periods (pH 4.5). Cellulase activity was lost after 3 min at 85°C.[22]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation and one batch produced for the toxicological tests (Table 1).[23] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation is 13.7% and the mean enzyme activity/TOS ratio is 271,461 CU/mg TOS.

Table 1: Composition of the food enzyme

Parameters	Unit	Batches			
		1	2	3	4[(a)]
Cellulase activity	CU/mg[(b)]	48,400	38,200	24,200	263,902
Protein	%	9.1	9.0	7.2	54.9
Ash	%	0.4	0.5	0.4	2.8
Water	%	86.0	86.4	85.1	7.0
Total organic solids (TOS)[(c)]	%	13.6	13.1	14.5	90.2
Activity/mg TOS	CU/mg TOS	355,882	291,603	166,897	292,574

(a): Batch used for the toxicological studies.
(b): CU: Cellulase Unit (see Section 3.3.1).
(c): TOS calculated as 100% – % water – % ash.

[16] Technical Dossier/Volume I/Annexes: 9, 11 and 12.
[17] Technical Dossier/Volume I/pp. 9/Annex 2.
[18] Technical Dossier/Volume I/Annex 2.
[19] Technical Dossier/Volume I/pp. 9/Annex 1.
[20] Technical Dossier/Volume I/pp. 14-15/Annexes: 3 and 4.
[21] Technical Dossier/Volume I/pp. 12/Annex 5.
[22] Technical Dossier/Volume I/pp. 12-14/Annex 6.
[23] Technical Dossier/Volume I/pp. 11, 41/Annexes: 3, 4, 13 and 14.

3.3.3. Purity

The lead content in the three commercial batches and in the batch used for toxicological studies was below 0.05 mg/kg[24] which complies with the specification for lead (≤ 5 mg/kg) 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 (LoDs) of the employed methods.[24,25]

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).[24]

Strains of *Trichoderma*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Frisvad et al., 2018). The presence of T-2 toxin and HT-2 toxin was examined in the three commercial food enzyme batches, and aflatoxins, fumonisins, ochratoxin A, sterigmatocystin, HT-2 toxin, T-2 toxin, zearalenone and deoxynivalenol in the batch used in toxicological testing. All were below the LoD of the applied methods.[24,26] Adverse effects caused by the possible presence of other secondary metabolites is 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 and DNA of the production strain

The absence of viable cells of the production strain in the food enzyme was demonstrated [redacted][27]

The absence of recombinant DNA in the food enzyme was demonstrated [redacted][28]

3.4. Toxicological data

A battery of toxicological tests including a bacterial gene mutation assay (Ames test), an *in vitro* micronucleus test and a repeated dose 90-day oral toxicity study in rats has been provided. The batch 4 (Table 1) used in these studies has a similar protein pattern and chemical purity as the batches used for commercialisation, and thus is considered suitable as a test item.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was performed according to the Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997) and following Good Laboratory Practice (GLP).[29] In a pre-experiment, two strains of *Salmonella* Typhimurium (TA98 and TA100) were tested at eight concentrations (corresponding to 3.16 to 5,000 μg TOS/plate) of the food enzyme in the presence or absence of metabolic activation (S9-mix) applying the plate incorporation method and with triplicate plating. No cytotoxicity or precipitation were seen at any concentration tested. Two main tests were then performed with five strains of *S*. Typhimurium (TA98, TA100, TA 102, TA1535 and TA1537), tested with six concentrations of the food enzyme corresponding to 31.6, 100, 316, 1,000, 2,500 and 5,000 μg TOS/plate, with or without S9-mix. In the first test, the plate incorporation method was applied and in the second test, the pre-incubation method was used. Upon treatment with the food enzyme, there was no relevant increase in revertant colony numbers above the control values in any strain with or without S9-mix, in either of the tests.

[24] Technical Dossier/Volume I/pp. 11–12, 41/Annexes: 3, 4, 13 and 14.
[25] LoDs: Pb, Cd and Hg = 0.025 mg/kg each; As = 0.25 mg/kg.
[26] LoDs: aflatoxins (B1, B2, G1 and G2) = 0.1 μg/kg each; fumonisins (B1, B2) = 5 μg/kg each; HT-2 toxin, T-2 toxin and sterigmatocystin = 10 μg/kg each; ochratoxin A = 2 μg/kg.
[27] Technical dossier/Volume II/Annexes 11 and 12 and Additional information February 2022/Annex 1.
[28] Technical dossier/Volume II/Annexes 13.
[29] Technical dossier/Volume I/Annex 13.

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

3.4.1.2. *In vitro* micronucleus assay

The *in vitro* micronucleus test was carried out according to OECD Draft Guideline 487 (OECD, 2010) and following GLP.[30] A cytotoxicity test and two main separate tests were performed in duplicate cultures of human peripheral whole blood lymphocytes. Cells were exposed to the test substance in a short-term-treatment (4 + 40 h recovery time) with or without metabolic activation (S9-mix). Additionally, a continuous 44-h treatment without S9-mix was included (44 + 0 h recovery time). In the cytotoxicity test, the food enzyme was tested at 10 concentrations (7.8–5,000 μg/mL). Based on the cytotoxicity test results, concentrations tested in the short-term treatments were 125, 250, 500, 1,000, 2,500 and 5,000 μg/mL (without S9-mix) and 125, 250, 500, 1,000, 2,000, 3,000, 4,000 and 5,000 μg/mL (with S9-mix). In the continuous treatment, concentrations of 25, 50, 125, 150, 175, 200, 225, 250 and 300 μg/mL (without S9-mix) were tested. Based on cytotoxicity observed in the main tests, concentrations of 250, 500 and 2,500 μg/mL (without S9-mix) and 500, 1,000, 2,000 and 4,000 μg/mL (with S9-mix) in the short-term treatment were selected for scoring of bi-nucleated cells with micronuclei (MNBN). In the continuous treatment, concentrations of 125, 175 and 200 μg/mL (without S9-mix) were scored for MNBN. The frequency of MNBN was comparable to the negative controls at all concentrations tested. The Panel concluded that the food enzyme did not induce an increase in the frequency of MNBN in cultured human peripheral blood lymphocytes under the test conditions employed in this study.

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.[31] Groups of 10 male and 10 female Wistar Crl: WI (Han) rats received by gavage the food enzyme at doses of 100, 300 or 1,000 mg TOS/kg bw per day. Controls received the vehicle (water for injection).

No mortality was observed.

The body weight gain was statistically significantly decreased at days 71–78 in mid- and high-dose females (−54%, −42%). The Panel considered the changes as not toxicologically relevant as they were without a statistically significant effect on the final body weight and body weight gain, they were only observed in one sex and at one time point, and there was no dose–response relationship.

The haematological investigation revealed a statistically significant increase in the mean corpuscular haemoglobin concentration in low-dose males (+4%) and in the percentage of eosinophils in mid-dose females (+61%). The Panel considered these changes as not toxicologically relevant as there was no dose–response relationship (both parameters) and they were only observed in one sex (both parameters).

The clinical chemistry investigation revealed a statistically significant decrease in aspartate aminotransferase (AST) in low- and high-dose females (−15%, −17%) and an increase in potassium in low- and mid-dose females (+9%, +17%). The Panel considered these changes as not toxicologically relevant as there was no dose–response relationship (both parameters) the magnitude of the changes was low (both parameters) and they were only observed in one sex (both parameters).

Statistically significant changes in hormone levels included a decrease in thyroid-stimulating hormone (TSH) in all treated female groups (−63%, −48%, −58%) and an increase in triiodothyronine (T3) in all treated female groups (+31%, +47%, +50%) and in TSH in mid-dose males (+95%). The Panel considered these changes as not toxicologically relevant as there was no dose–response relationship (TSH), they were only observed in one sex (T3), there was no consistency between the change in males and females (TSH) and the concurrent control values were at the upper end of the historical control values.

Statistically significant changes in organ weights included an increase in the absolute and relative (to brain) spleen weight in mid-dose males (+18% and + 20%, respectively) and in relative (to body) spleen weight in mid-dose females (+14%). The Panel considered the changes as not toxicologically relevant as there was no dose–response relationship.

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

[30] Technical dossier/Volume I/Annex 14.
[31] Technical dossier/Volume I/Annex 15 and Additional information February 2022/Annex 3.

The Panel identified the no observed adverse effect level (NOAEL) of 1,000 mg TOS/kg bw per day, the highest dose tested.

3.4.3. 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 cellulase produced with the genetically modified *T. reesei* strain AR-852 was assessed by comparing its amino acid sequence with those of known allergens according to the 'Scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms' (EFSA GMO Panel, 2010). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, no match was found.[32]

No information is available on oral and respiratory sensitisation or elicitation reactions of this cellulase.

Respiratory allergic reactions following occupational inhalation of cellulase have been reported (Elms et al., 2003; Martel et al., 2010). However, some studies have shown that adults with occupational asthma to an enzyme used in food can commonly ingest the corresponding allergen without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). Information on adverse reactions upon ingestion of cellulase in individuals sensitised through the respiratory route has not been reported.

A substance that may cause allergies or intolerances (listed in Regulation (EU) No 1169/2011[33]) is used as raw material ([redacted]). In addition, [redacted] a known source of allergens, is also present in the media fed to the microorganisms. However, during the fermentation process, these products will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the fungal 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 (except for distilled alcohol production), 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 five food processes at the recommended use levels summarised in Table 2.

Table 2: Intended uses and recommended use levels of the food enzyme as provided by the applicant[(c)]

Food manufacturing process[(a)]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[(b)]
Baking processes	Flour	**2**
Brewing processes	Cereals	**2**
Production of distilled alcohol	Cereals	2
Wine and wine vinegar production	Grapes	**2**

[32] Technical Dossier/Volume I/pp. 42-44/Annex 2.

[33] 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.

Food manufacturing process[(a)]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[(b)]
Fruit and vegetable processing for juice production	Fruit/vegetables	**2**

(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): The numbers in bold were used for calculations.
(c): Technical dossier/p. 30 and Additional data February 2022.

In baking processes, cellulase is added to the flour to make dough or batter.[34] The enzymatic treatment improves dough handling and reduces batter viscosity. The food enzyme remains in the final foods.

In brewing processes, cellulase is added to the cereals during the mashing step. It can be added also to the wort during fermentation.[35] The enzymatic treatment can increase the choice of raw material, facilitates filtration by reducing viscosity, reduces haze and turbidity. The food enzyme remains in the beer.

In the production of distilled alcohol, cellulase is added to the cereals during mixing and liquefaction before the fermentation.[36] The enzymatic treatment decreases viscosity, improves processing and increases yield. The food enzyme is not carried over with the distilled alcohols (EFSA CEP Panel, 2021a).

In wine and wine vinegar production, cellulase is added to grapes during crushing contributing to the maceration.[37] The enzymatic treatment increases the release of must, eases pressing and increases yield. The food enzyme remains in the final foods.

In fruit and vegetable processing for juice production, cellulase is added during peeling and crushing.[38] The enzymatic treatment decreases viscosity thereby easing pressing and releasing of cell contents. The food enzyme remains in juices.

Based on the temperature profile of the food enzyme (see Section 3.3.1), it is expected that the food enzyme is inactivated in all the food processes except wine and wine vinegar production.

3.5.2. Dietary exposure estimation

A dietary exposure was calculated only for food manufacturing processes where the food enzyme–TOS remains in the final foods, namely baking, brewing processes, wine and wine vinegar production, and fruit and vegetable processing for juice production.

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

Table 3 provides an overview of the derived exposure estimates across all surveys. Detailed average and 95th percentile exposure to the food enzyme–TOS per age class, country and survey, as well as contribution from each FoodEx category to the total dietary exposure are reported in Appendix A – Tables 1 and 2. For the present assessment, food consumption data were available from 41 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). The highest dietary exposure to the food enzyme–TOS was estimated to be about 0.096 mg TOS/kg bw per day in the population groups of toddlers and children (between 1 and 9 years old).

[34] Technical dossier/p. 31.
[35] Technical dossier/p. 32.
[36] Technical dossier/p. 33.
[37] Technical dossier/p. 34.
[38] Technical dossier/p. 35.

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)	0.001–0.018 (11)	0.008–0.060 (15)	0.009–0.034 (19)	0.006–0.021 (21)	0.005–0.015 (22)	0.004–0.013 (22)
Min–max 95th percentile (number of surveys)	0.005–0.057 (9)	0.025–0.096 (13)	0.017–0.096 (19)	0.013–0.056 (20)	0.013–0.045 (22)	0.011–0.032 (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, 2007), 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	
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 other processes from the exposure assessment – Production of distilled alcohol	–

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 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.

The exclusion of one food manufacturing process 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

A comparison of the NOAEL (1,000 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0.001–0.060 mg TOS/kg bw per day at the mean and from 0.005–0.096 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposure of at least 10,417.

4. Conclusions

Based on the data provided, the removal of TOS during distilled alcohol production and the derived margin of exposure for the other intended uses, the Panel concludes that the food enzyme cellulase produced with the genetically modified *Trichoderma reesei* strain AR-852 does not give rise to safety concerns under the intended conditions of use.

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

5. Documentation as provided to EFSA

Application for authorisation of a cellulase from a genetically modified strain of *Trichoderma reesei*. August 2021. Submitted by AB Enzymes GmbH.

Additional information. February 2022. Submitted by AB Enzymes GmbH.

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Abbreviations

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
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	limit of detection
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	total organic solids
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.2022.7375#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).