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Safety evaluation of the food enzyme endo-1,3(4)-β-glucanase from the non-genetically modified *Rasamsonia composticola* strain 427-FS

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Abstract

The food enzyme endo-1,3(4)-β-glucanase (3-(1–3,1–4)-β-D-glucan 3(4)-glucanohydrolase; EC 3.2.1.6) is produced with the non-genetically modified *Rasamsonia composticola* 427-FS strain by Kerry Ingredients & Flavours Ltd. The food enzyme is free from viable cells of the production organism. The food enzyme is intended to be used in six manufacturing processes, i.e. baking processes, other cereal-based processes, brewing processes, grain treatment for the production of starch and gluten fractions, distilled alcohol production and yeast processing. Since residual amounts of total organic solids (TOS) are removed by distillation and during grain processing, dietary exposure was calculated only for the remaining four processes. It was estimated to be up to 0.809 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 866 mg TOS/kg bw per day, the highest dose tested, which when compared with the estimated dietary exposure, resulted in a margin of exposure of at least 1,070. A search for the 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, 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: food enzyme, endo-1,3(4)-β-glucanase, 3-(1→3;1→4)-β-D-glucan 3(4)-glucanohydrolase, EC 3.2.1.6, laminarinase, *Rasamsonia composticola*

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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 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.

The following three applications have been submitted for the authorisation of food enzymes:

1) From "Amano Enzyme Inc." for Alpha-glucosidase from *Aspergillus niger* (strain AE-TGU);
2) From the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) for Endo-1,3(4)-β-glucanase, Endo-1,4-β-xylanase and Cellulase from *Talaromyces emersonii*;
3) From AMFEP for Cellulase, Endo-1,3(4)-β-glucanase and Endo-1,4-β-xylanase obtained from *Trichoderma reesei*.

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] 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.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes Alpha-glucosidase from *Aspergillus niger* (strain AE-TGU), Endo-1,3(4)-β-glucanase, Endo-1,4-β-xylanase and Cellulase from *Talaromyces emersonii*, and Cellulase, Endo-1,3(4)-β-glucanase and Endo-1,4-β-xylanase obtained from *Trichoderma reesei* in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of the food enzyme endo-1,3(4)-β-glucanase, endo-1,4-β-xylanase and cellulase from *Talaromyces emersonii* submitted by AMFEP.

The application was submitted initially as a joint dossier[4] and identified as the EFSA-Q-2014-00801, EFSA-Q-2014-00802 and EFSA-Q-2014-00803. During the risk assessment phase, it was found that the technical dossier is too generic to be evaluated. A solution was found in 16 March 2020 via an ad hoc meeting between EFSA, the European Commission and representatives from the Association of Manufacturers and Formulators of Enzyme Products (AMFEP).[5] It was agreed that the joint dossier will be split into individual data packages.

The current opinion addresses one data package originating from the joint dossier EFSA-Q-2014-00801, EFSA-Q-2014-00802 and EFSA-Q-2014-00803. This data package, identified as EFSA-Q-2021-00746, concerns the food enzyme produced with *Talaromyces emersonii* strain 427-FS and submitted by Kerry Ingredients & Flavours Ltd.

Recent data identified the production microorganism as *Rasamsonia composticola* (Section 3.1). Therefore, this name will be used in this opinion instead of *Talaromyces emersonii*.

2. Data and Methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme endo-1,3(4)-β-glucanase from *Rasamsonia composticola*.

Additional information was requested from the applicant during the assessment process on 17 March 2022 and received on 16 September 2022 (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 documents of EFSA Scientific Committee.

The 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA, 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 with the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021a).

3. Assessment

IUBMB nomenclature	Endo-1,3(4)-β-glucanase
Systematic name	3-(1→3;1→4)-β-D-Glucan 3(4)-glucanohydrolase
Synonyms	Endo-1,3-β-D-glucanase; laminarinase; β-1,3-glucanase
IUBMB No	3.2.1.6
CAS No	62213-14-3
EINECS No	263-462-4

[4] Commission Implementing Regulation (EU) No 562/2012 of 27 June 2012 amending Commission Regulation (EU) No 234/2011 with regard to specific data required for risk assessment of food enzymes Text with EEA relevanceOJ L 168, 28.6.2012, pp. 21–23.

[5] The full detail is available at the https://www.efsa.europa.eu/en/events/event/ad-hoc-meeting-industry-association-amfep-joint-dossiers-food-enzymes

Endo-1,3(4)-β-glucanases catalyse the hydrolysis of 1,3- and 1–4-β-glycosidic linkages in mixed-linked β-D-glucans resulting in the generation of partially hydrolysed β-D-glucans. The food enzyme is intended to be used in six food manufacturing processes: baking processes, cereal-based processes, brewing processes, grain treatment for the production of starch and gluten fractions, distilled alcohol production and yeast processing.

3.1. Source of the food enzyme

The endo-1,3(4)-β-glucanase is produced with the non-genetically modified filamentous fungus *R. composticola* strain 427-FS (formerly *Talaromyces emersonii*), which is deposited at the Westerdijk Fungal Biodiversity Institute culture collection (CBS, the Netherlands) with the deposit number CBS [redacted].[6] The production strain was identified as *R. composticola* by phylogenomic analysis using the production strain whole genome sequence (WGS) and sequence analysis of the D2 region of the ribosomal large subunit.[7]

The genome of *R. composticola* 427-FS was searched for gene clusters with known functions in the synthesis of compounds with known toxicity and none were found.[7]

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[8], with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[9]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, batch or 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 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.[10] 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.[11]

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 endo-1,3(4)-β-glucanase is a single polypeptide chain of [redacted] amino acids as derived from the WGS. The molecular mass of the mature protein, calculated from the amino acid sequence, was [redacted] kDa. The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). A consistent protein pattern was observed across all batches. The gels showed a band with a mass of 42 kDa in all batches consistent with the calculated mass of the food enzyme. The protein profile also included many bands of equal or lesser staining intensity.[12] The food enzyme contains xylanase activity.[13]

The in-house determination of endo-1,3(4)-β-glucanase activity is based on hydrolysis of β-D-glucan (reaction conditions: pH 5.0, 50°C, 10 min). The enzymatic activity is determined by measuring the release of reducing groups that, in the presence of 3,5-dinitrosalicylic acid, form a coloured product detected spectrophotometrically at 540 nm. The enzyme activity is expressed in Units/mL (U/mL). One Unit is defined as the amount of enzyme, which will produce 1 mg of reducing sugar (maltose equivalents) under the assay conditions.[14]

[6] Technical dossier/Additional information September 2022/Annex XVIII.
[7] Technical dossier/Annex VIII.
[8] 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.
[9] Technical dossier/p. 39.
[10] Technical dossier/pp. 38–44.
[11] Technical dossier/Annex 11.
[12] Technical dossier/Additional information September 2022/Annex 21.
[13] Technical dossier/p. 24.
[14] Technical dossier/Annex 2.1.

The food enzyme has a temperature optimum around 60°C (pH 5.0) and a pH optimum around pH 5.5 (50°C). Thermostability was tested after a pre-incubation of the food enzyme for 15 min at different temperatures. Activity was unaffected by temperatures up to 65°C but was lost at 80°C.[15]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation and three batches produced for the toxicological tests (Table 1).[16] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation is 15.3% and the mean enzyme activity/TOS ratio was 44.5 Units/mg TOS.

Table 1: Composition of the food enzyme

Parameters	Unit	Batches					
		1	2	3	4[(a)]	5[(b)]	6[(c)]
Endo-1,3(4)-β-glucanase activity	U/g[(d)]	7,002	6,610	6,518	38,437	33,529	7,334
Protein	%	9.6	9.5	10.4	78.4	63.5	12
Ash	%	0.6	0.4	0.2	1.9	2.6	0.6
Water	%	86.0	83.0	84.0	7.8	10.8	84.2
Total organic solids (TOS)[(e)]	%	13.4	16.6	15.8	90.3	86.6	15.2
Endo-1,3(4)-β-glucanase activity/TOS	U/mg TOS	52.3	39.8	41.3	42.6	38.7	48.9

(a): Batch used for the Ames test and chromosomal aberration test.
(b): Batch used for the repeated dose 90-day oral toxicity study in rats.
(c): Batch used for the micronucleus test.
(d): U: Unit (see Section 3.3.1).
(e): TOS calculated as 100% - % water - % ash.

3.3.3. Purity

The lead content in the three commercial batches was < 0.005 mg/kg,[17] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[18]

The food enzyme complies with the microbiological criteria (for total coliforms, *Escherichia coli* and *Salmonella*)[19] 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 *Rasamsonia* (formerly *Talaromyces*), in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Samson et al., 2011). The possible presence of secondary metabolites of concern 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 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 hundred millilitres of product was filtered through a 0.45-μm pad, the pad was impregnated with non-selective medium and incubated at 44°C for 7 days. No colonies were produced. A positive control was included.[22]

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, was provided.

[15] Technical dossier/Annex 28.
[16] Technical dossier/p. 24 and Annexes 1,13,14,15.
[17] Technical dossier/p. 26 and Annex 1.
[18] Limit of detection (LoD): Pb = 0.005 mg/kg.
[19] Technical dossier/Annexes 1, 13, 15.
[20] Technical dossier/Annex 26.
[21] Technical dossier/Annexes 1 and 3.
[22] Technical dossier/Annex 7.

The batches 4, 5 and 6 (Table 1) used in these studies have similar activity/TOS values as the batches used for commercialisation and are considered suitable as test items.

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, 1997) and following Good Laboratory Practice (GLP).[23]

Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2uvrA(pKM101) were used with or without metabolic activation (S9-mix). Two experiments were carried out in triplicate. The first experiment applied the plate incorporation method, using seven concentrations of the food enzyme ranging from 50 to 5,000 μg/plate (corresponding to 45 to 4,515 μg TOS/plate). The second experiment applied the pre-incubation method using five concentrations of the food enzyme ranging from 50 to 5,000 μg/plate (corresponding to 45 to 4,515 μg TOS/plate).

No cytotoxicity was observed at any concentration tested of the food enzyme. Upon treatment with the food enzyme, there were no biologically relevant increases in the number of revertant colonies above the control values in any of the strains tested, with or without S9-mix.

The Panel concluded that the food enzyme endo-1,3(4)-β-glucanase 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, 2016a,b) and following GLP.[24]

An experiment was performed with duplicate cultures of human peripheral whole blood lymphocytes. The cell cultures were treated with the food enzyme either with or without metabolic activation (S9-mix). In a range-finding test where cells were treated with ten concentrations ranging from 20.16 to 2,000 μg/mL (corresponding to 18–1,806 μg TOS/mL), no cytotoxicity above 50% was seen. The cells were exposed to the food enzyme and scored for chromosomal aberrations at three concentrations of 720, 1,200 and 2,000 μg/mL (corresponding to 650, 1,084 and 1,806 μg TOS/mL), in a short-term treatment (3 h exposure and 18 h recovery period) either with or without S9-mix and in a long-term treatment (21 h exposure with no recovery period) without S9-mix.

No cytotoxicity based on the reduction of the mitotic index (MI) was seen either in the short-term (with or without S9-mix) or in the long-term treatment. The frequency of structural and numerical aberrations was not statistically significantly different to the negative controls at all concentrations tested.

The Panel concluded that the food enzyme endo-1,3(4)-β-glucanase did not induce an increase in the frequency of structural and numerical 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, 2016a,b) and following GLP.[25]

An experiment was performed with duplicate cultures of Chinese hamster ovary cells (GHO-K1). The cell cultures were treated with the food enzyme and scored for binucleated cells with micronuclei (MNBN) at concentrations of 1,200, 2,500 and 5,000 μg TOS/mL in short-term treatments (4 h treatment followed by 20 h recovery) with or without metabolic activation (S9-mix) and at 120, 250 or 500 μg TOS/mL in a long-term treatment (24 h treatment without recovery) without S9-mix.

In the short-term treatments, no cytotoxicity above 50% was seen at any concentration tested up to 5,000 μg TOS/mL with or without metabolic activation (S9-mix). In the long-term treatment, cytotoxicity of 47% was seen at 500 μg TOS/mL. The frequency of MNBN was not statistically significantly different to the negative controls at any concentrations in any of the treatments and all results were within the 95% historical control range.

[23] Technical dossier/p. 56 and Annex 13.
[24] Technical dossier/p. 56 and Annex 14.
[25] Technical dossier/Additional information September 2022/Annex 14.

The Panel concluded that the food enzyme endo-1,3(4)-β-glucanase did not induce an increase in the frequency of MNBNs under the test conditions applied 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 following GLP[26] and in accordance with OECD Test Guideline 408 (OECD, 2018) with the following deviation: urea was not determined. The Panel considered that this deviation is minor and does not impact on the evaluation of the study.

Groups of 10 male and 10 female Sprague–Dawley (NTac:SD) rats received by gavage the food enzyme in doses of 250, 500 or 1,000 mg/kg body weight (bw) per day corresponding to 217, 433 or 866 mg TOS/kg bw per day. Controls received the vehicle (ultrapure water).

No mortality was observed.

The gain was statistically significantly increased on days 1–8 of administration in high-dose females (+30%). The Panel considered the change as not toxicologically relevant as it was only recorded sporadically, as it was only observed in one sex and because the change was without a statistically significant effect on the final body weight and the final body weight gain.

The haematological investigation revealed a statistically significant increase in mean corpuscular haemoglobin (MCH) in low- and high-dose females (+6% and +4%, respectively), an increase in mean corpuscular volume (MCV) in all treated female groups (+5%, +4% and +4%, respectively), a decrease in mean corpuscular haemoglobin concentration (MCHC) in low- and high-dose males (−2% and −2%, respectively), an increase in neutrophils in high-dose females (+40%) and a decrease in prothrombin time in high-dose females (−9%). The Panel considered the changes as not toxicologically relevant because they were only observed in one sex (all parameters), there was no dose–response relationship (MCH, MCV, MCHC), the changes were small (all parameters except neutrophils), there were no changes in other relevant parameters (for MCH, MCV, MCHC in the red blood cell count, haemoglobin and haematocrit; for neutrophils in the total white blood cell count) and there were no histopathological changes in the bone marrow, spleen, liver or lymph nodes.

The clinical chemistry investigation revealed a statistically significant increase in total protein in high-dose males (+5%), an increase in globulin in high-dose males (+5%), an increase in chloride in high-dose females (+2%), a decrease in total bilirubin in high-dose males (−67%), a decrease in calcium in high-dose males (−6%), a decrease in phosphorus in mid- and high-dose males (−8% and −7%, respectively) and a decrease in sodium in mid- and high-dose males (−1% and −2%, respectively). The Panel considered the changes as not toxicologically relevant because they were only observed in one sex (all parameters), there was no dose–response relationship (phosphorus) and the changes were small (all parameters except bilirubin).

The urinalysis revealed a statistically significant increase in the urine volume in mid- and high- dose males (+158% and +270%, respectively) and an increase in the specific gravity in mid-dose females (+1%). The Panel considered the changes as not toxicologically relevant because they were only observed in one sex (both parameters), there was no dose–response relationship (specific gravity), the change was small (specific gravity), there were no changes in other relevant parameters and there were no histopathological changes in kidneys.

Statistically significant changes in organ weights included an increase in the absolute thyroid and parathyroid weight in high-dose males (+24%) and in low-dose females (+28%). The Panel considered the changes as not toxicologically relevant because there was no dose–response relationship (females), there was no change in the relative weight and there were no histopathological changes in the thyroid/parathyroid.

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

The Panel identified the no observed adverse effect level (NOAEL) of 866 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity

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

The potential allergenicity of the endo-1,3(4)-β-glucanase produced with the non-genetically modified *Rasamsonia composticola* strain 427-FS was assessed by comparing its amino acid sequence with those of known allergens according to the 'Scientific opinion on the assessment of allergenicity of

[26] Technical dossier/pp. 60–63 and Annex 15.

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.[27]

No information is available on oral and respiratory sensitisation or elicitation reactions of this endo-1,3(4)-β-glucanase.

While several studies report positive IgE-reactivity of β-1,3-glucanases derived from plant and dust mites upon respiratory exposure, studies have shown that adults respiratory sensitised can ingest the allergens without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Poulsen, 2004; Armentia et al., 2009). Furthermore, no allergic reactions upon dietary exposure to any β-glucanase have been reported in the literature.[28]

[redacted] a product that may cause allergies (listed in the Regulation (EU) No 1169/2011[29]), is used as a raw material. In addition, [redacted] and [redacted], known sources of allergens, are 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 no potentially allergenic residues are present in the food enzyme.

The Panel considered that, under the intended conditions of use, 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 six 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[30]

Food manufacturing process[(a)]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[(b)]
Baking processes	Flour	17–**50**
Cereal-based processes	Flour	0.1–**100**
Brewing processes	Cereals	0.6–**50**
Grain treatment for the production of starch and gluten fractions	Cereals	4–75
Distilled alcohol production	Cereals	5–20
Yeast processing	Yeast biomass, autolysed yeast, yeast extract, cell walls	10–**250**

(a): The name has been harmonised by EFSA 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 calculation.

In baking and cereal-based processes, the food enzyme is added to flour during the preparation of the dough or batter.[31] The endo-1,3(4)-β-glucanase hydrolyses β-D-glucans, increasing the water-binding capacity of the dough and reducing viscosity. The food enzyme–TOS remains in the final food products.

[27] Technical dossier/pp. 64–66 and Annex 17.1.
[28] Technical dossier/Annex 17.2.
[29] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.
[30] Technical dossier/p. 51 & Additional information September 2022/Answer 8.
[31] Technical dossier/Additional information September 2022/Annex 12 v2.

In brewing process, the food enzyme is added to cereals during the mashing step.[32] The endo-1,3(4)-β-glucanase degrades cell wall glucans, promoting the release of starch and protein and increasing the brewing yield. The food enzyme is also added during fermentation to aid beer filtration. The food enzyme–TOS remains in beer.

In grain treatment, the food enzyme can be added to the grain to obtain flour, or to the dough to obtain starch and gluten fractions.[32] The food enzyme–TOS is removed in the final processed foods by repeated washing and purification steps applied during grain treatment (EFSA CEP Panel, 2021b).

In distilled alcohol production, the food enzyme is applied during liquefaction and fermentation and may also be added during slurry mixing and pre-saccharification.[32] The food enzyme–TOS is not carried over with the distilled alcohols (EFSA CEP Panel, 2021b).

In yeast processing, the food enzyme can be added to yeast biomass, autolysed yeasts, yeast extracts or yeast cell walls.[33] The endo-1,3(4)-β-glucanase degrades cell wall glucans, improving the extraction process of cellular components from yeast. The food enzyme–TOS remains in different yeast products.

Based on data provided on thermostability (see Section 3.3.1), it is expected that the endo-1,3(4)-β-glucanase will be inactivated during all the food manufacturing processes.

3.5.2. Dietary exposure estimation

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

Chronic exposure to the food enzyme–TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSA CEP Panel, 2021a). The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP 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 mean and 95th percentile exposure to the food enzyme–TOS per age class, country and survey, as well as contribution from each FoodEx category to the total dietary exposure are reported in Appendix A – Tables 1 and 2. For the present assessment, food consumption data were available from 41 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). The highest dietary exposure was estimated to be 0.809 mg TOS/kg bw per day in toddlers at the 95th percentile.

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)	0.042–0.211 (11)	0.201–0.553 (15)	0.213–0.445 (19)	0.107–0.264 (21)	0.071–0.181 (22)	0.063–0.167 (22)
Min–max 95th percentile (number of surveys)	0.223–0.596 (9)	0.403–0.809 (13)	0.372–0.782 (19)	0.200–0.554 (20)	0.162–0.419 (22)	0.128–0.290 (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.

[32] Technical dossier/Annex 12.
[33] Technical dossier/Annex 13–2.

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 was 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	+/–
Exclusion of other processes from the exposure assessment – Grain treatment for the production of starch and gluten fractions – Distilled alcohol production	–

TOS: total organic solids.
+: Uncertainty with potential to cause overestimation of exposure.
–: Uncertainty with potential to cause underestimation of exposure.

The conservative approach applied to the exposure estimate of 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 two food manufacturing processes 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 (866 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0.042–0.553 mg TOS/kg bw per day at the mean and from 0.128–0.809 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposure (MoE) of at least 1,070.

4. Conclusions

Based on the data provided on removal of TOS during the two food manufacturing processes and the derived margin of exposure for the remaining four food processes, the Panel concluded that the food enzyme endo-1,3(4)-β-glucanase produced with *R. composticola* strain 427-FS does not give rise to safety concerns under the intended conditions of use.

5. Documentation as provided to EFSA

Technical dossier 'APPLICATION FOR AUTHORISATION OF USE OF Endo-1,3(4)-β-glucanase FROM *Rasamsonia composticola* IN ACCORDANCE WITH REGULATION (EC) 1331/2008' December 2021. Submitted by Kerry Ingredients & Flavours Ltd.

Additional information. September 2022. Submitted by Kerry Ingredients & Flavours Ltd.

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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
MoE	margin of exposure
OECD	Organisation for Economic Cooperation and Development
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	total organic solids
WGS	whole genome sequencing
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 at https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2023.7751#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).