

ADOPTED: 7 July 2022

doi: 10.2903/j.efsa.2022.7457

Safety evaluation of the food enzyme mucorpepsin from *Rhizomucor miehei* strain DSM 29547

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (EFSA CEP Panel), Claude Lambré, José Manuel Barat Baviera, Claudia Bolognesi, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis, Holger Zorn, Lieve Herman, Joaquim Maia, Natalia Kovalkovicova, Simone Lunardi, Yi Liu and Andrew Chesson

Abstract

The food enzyme mucorpepsin (EC 3.4.23.23) is produced with the non-genetically modified *Rhizomucor miehei* strain DSM 29547 by Chr. Hansen. The food enzyme is free from viable cells of the production organism. It is intended to be used in dairy processing for cheese production. The dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.26 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 a repeated dose 90-day oral toxicity study in rats. The Panel identified a no observed adverse effect level of 618 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 2,400. A search for similarity of the amino acid sequence of the food enzyme to known allergens was made and three matches were found. The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded but is considered low except for individuals sensitised to mustard proteins, but this risk will not exceed that of mustard consumption. 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.

© 2022 Wiley-VCH Verlag GmbH & Co. KGaA on behalf of the European Food Safety Authority.

Keywords: food enzyme, mucorpepsin, aspartic endopeptidase, EC 3.4.23.23, microbial rennet, *Rhizomucor miehei*

Requestor: European Commission

Question number: EFSA-Q-2015-00761

Correspondence: fip@efsa.europa.eu

Panel members: José Manuel Barat Baviera, Claudia Bolognesi, Andrew Chesson, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Claude Lambré, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis and Holger Zorn.

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.

Declarations of interest: If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

Suggested citation: EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré C, Barat Baviera JM, Bolognesi C, Chesson A, 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, Herman L, Maia J, Kovalkovicova N, Lunardi S, Liu Y and Chesson A, 2022. Scientific Opinion on the safety evaluation of the food enzyme mucorpepsin from *Rhizomucor miehei* strain DSM 29547. EFSA Journal 2022;20(8):7457, 14 pp. <https://doi.org/10.2903/j.efsa.2022.7457>

ISSN: 1831-4732

© 2022 Wiley-VCH Verlag GmbH & Co. KGaA on behalf of the European Food Safety Authority.

This is an open access article under the terms of the [Creative Commons Attribution-NoDerivs](https://creativecommons.org/licenses/by-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited and no modifications or adaptations are made.



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



Table of contents

Abstract.....	1
1. Introduction.....	4
1.1. Background and Terms of Reference as provided by the requestor.....	4
1.1.1. Background as provided by the European Commission.....	4
1.1.2. Terms of Reference.....	5
1.2. Interpretation of the Terms of Reference.....	5
2. Data and methodologies.....	5
2.1. Data.....	5
2.2. Methodologies.....	5
3. Assessment.....	5
3.1. Source of the food enzyme.....	5
3.2. Production of the food enzyme.....	6
3.3. Characteristics of the food enzyme.....	6
3.3.1. Properties of the food enzyme.....	6
3.3.2. Chemical parameters.....	7
3.3.3. Purity.....	7
3.3.4. Viable cells of the production strain.....	7
3.4. Toxicological data.....	8
3.4.1. Genotoxicity.....	8
3.4.1.1. Bacterial reverse mutation test.....	8
3.4.1.2. <i>In vitro</i> mammalian chromosomal aberration test.....	8
3.4.2. Repeated dose 90-day oral toxicity study rodents.....	8
3.4.3. Allergenicity.....	9
3.5. Dietary exposure.....	10
3.5.1. Intended use of the food enzyme.....	10
3.5.2. Dietary exposure estimation.....	10
3.5.3. Uncertainty analysis.....	10
3.6. Margin of exposure.....	11
4. Conclusions.....	11
5. Documentation as provided to EFSA.....	11
References.....	11
Abbreviations.....	12
Appendix A – Dietary exposure estimates to the food enzyme–TOS in details.....	13
Appendix B – Population groups considered for the exposure assessment.....	14

1. Introduction

Article 3 of the Regulation (EC) No 1332/2008¹ 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 1331/2008² 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.

Six applications have been introduced by the companies "Amano Enzyme Inc." for the authorization of the food enzyme Alpha-L-rhamnosidase from *Penicillium decumbens* (strain AE-HP) and Acylglycerol lipase from a genetically modified strain of *Penicillium camemberti* (strain AE-LGS), and "Chr. Hansen" for the authorization of the food enzymes Chymosin from a genetically modified strain of *Aspergillus niger* var. awamori (strain DSM 29544), Chymosin from a genetically modified strain of *Aspergillus niger* var. awamori (strain DSM 29545), Chymosin from a genetically modified strain of *Aspergillus niger* var. awamori (strain DSM 29546) and Mucorpepsin from *Rhizomucor miehei* (strain DSM 29547).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011³ implementing Regulation (EC) No 1331/2008, the Commission has verified that the six 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 of the food enzymes Alpha-L-rhamnosidase from *Penicillium decumbens* (strain AE-HP), Acylglycerol lipase from a genetically modified strain of *Penicillium camemberti* (strain AE-LGS), Chymosin from a genetically modified strain of *Aspergillus niger* var. *awamori* (strain DSM 29544), Chymosin from a genetically modified strain of *Aspergillus niger* var. *awamori* (strain DSM 29545), Chymosin from a genetically modified strain of *Aspergillus niger* var. *awamori* (strain DSM 29546) and Mucorpepsin from *Rhizomucor miehei* (strain DSM 29547) 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 food enzyme mucorpepsin from *R. miehei* (strain DSM 29547).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme mucorpepsin from a non-genetically modified *R. miehei* (strain DSM 29547).

Additional information was spontaneously provided from the applicant on 23 January 2019.

Additional information was requested from the applicant during the assessment process on 06 July 2020 and received on 17 December 2020 (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 guidance documents of the 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	Mucorpepsin
Systematic name	Aspartic endopeptidase
Synonyms	Microbial rennet, Mucor rennin
IUBMB no	EC 3.4.23.23
CAS no	148465-73-0
EINECS no	642-981-3

Mucorpepsins catalyse the hydrolysis of proteins, including the peptide bond Phe105-Met106 of κ -casein in milk, resulting in the destabilisation of casein micelles and causing milk to clot. The food enzyme is intended to be used in dairy processing for cheese production.

3.1. Source of the food enzyme

The mucorpepsin is produced with the filamentous fungus *Rhizomucor miehei* strain DSM 29547, which is deposited at the German Collection of Microorganisms and Cell Cultures GmbH (DSMZ, Germany), with deposit number DSM 29547.⁴

The production strain was derived from the parental strain [REDACTED]. The parental strain was identified as

⁴ Technical dossier/Annex 11.

R. miehei by sequence analysis of the internal transcribed spacer (ITS) and the 28s rRNA gene and comparing these sequences with those in GenBank and in the fungal database of Westerdijk Fungal Biodiversity Institute, which contain sequences of most of the type strains.⁵

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004⁶, with food safety procedures based on hazard analysis and critical control points, and in accordance with current good manufacturing practice.⁷

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. Deglycosylation is applied to the food enzyme as part of the production process using an endo- β -*N*-acetylglucosaminidase (EC 3.2.1.96) to increase the milk clotting activity.

The food enzyme concentrate may be used in an unmodified form or may be treated with peracetic acid or hydrogen peroxide to make the enzyme more heat labile.⁸

The food enzyme is then filtered and formulated.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 mature mucorpepsin is a single polypeptide chain of 361 amino acids. The molecular mass of the mature protein, calculated from the amino acid sequence, is around 38.7 kDa.¹¹ The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis. A consistent protein pattern was observed across all batches. The gels showed a single major protein band corresponding to an apparent molecular mass of about 41 kDa, consistent with the expected mass of the enzyme.¹² The food enzyme was tested for lipase and amylase activities and neither were detected.¹³ No other enzymatic activities were reported.

The determination of mucorpepsin activity is based on the hydrolysis of casein resulting in milk clotting (reaction conditions: pH 6.5, 32°C). The enzymatic activity is determined by measuring the time needed for visual flocculation of a standard milk substrate. The mucorpepsin activity is quantified relative to an internal enzyme standard and expressed in International Milk Clotting Units/g (IMCU/g).¹⁴

The thermostable food enzyme has a temperature optimum around 55°C, the highest temperature tested (pH 6.5), while the thermolabile form of the food enzyme has a temperature optimum between 40 and 45°C (pH 6.5). Both forms of the food enzyme have a pH optimum around pH 6.3 (32°C), the lowest pH value tested.¹⁵ The activity of the thermolabile form of the food enzyme decreased by 98% after 15 s of incubation at 72°C (pH 6.0) or by 99% after 5 min at 60°C (pH 6.0).¹⁶

⁵ Technical dossier/Additional data December 2020/Annex Q1.

⁶ 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.

⁷ Technical dossier/Annexes 17 and 18.

⁸ Technical dossier/Additional data December 2020/Annex Q4.

⁹ Technical dossier/Section 3.2.1.2.5; Annexes 16, 19 and 20.

¹⁰ Technical dossier/Additional data December 2020/Annex Q5.

¹¹ Technical dossier/Additional data December 2020/Annex Q7.

¹² Technical dossier/Additional data December 2020/Annex Q6.

¹³ Technical dossier/Annexes 8–10.

¹⁴ Technical dossier/Annex 7.

¹⁵ Technical dossier/p. 37–38.

¹⁶ Technical dossier/p. 39.

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).¹⁷ The mean total organic solids (TOS) of the three food enzyme batches for commercialisation is 4.6% and the mean enzyme activity/TOS ratio is 50.6 IMCU/mg TOS.

Table 1: Compositional data of the food enzyme

Parameters	Unit	Batches			
		1	2	3	4 ^(a)
Mucorpepsin activity	IMCU/g batch ^(b)	2,363	2,311	2,282	3,190
Protein	%	3.9	3.5	3.5	3.6
Ash	%	9.7	9.4	9.7	< 0.1
Water	%	85.3	86.2	85.9	94.7
Total organic solids (TOS)^(c)	%	5.0	4.4	4.4	5.3
Activity/mg TOS	IMCU/mg TOS	47.3	52.5	51.9	60.2

(a): Batch used for the toxicological studies.

(b): IMCU: International Milk Clotting Units.

(c): TOS calculated as 100% – % water – % ash.

3.3.3. Purity

The lead content in the three commercial batches was below 5 mg/kg¹³ which complies with the specification for lead (≤ 5 mg/kg) as laid down in the general specifications and considerations 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 methodologies.^{13,18}

The food enzyme preparation complies with the microbiological criteria (for total coliforms, *Escherichia coli* and *Salmonella*)¹³ as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). Antimicrobial activity was detected against *Serratia marcescens* in one of the three batches tested.¹⁹ This result was considered incidental.

The presence of secondary metabolites was not examined in the food enzyme. Instead, the applicant provided data demonstrating that the production strain does not produce any known secondary metabolites when grown on four different media and screened by liquid chromatography-diode array detection-time of flight/mass spectrometry (LC-DAD-TOF/MS).²⁰ The possible presence of other secondary metabolites of concern in the food enzyme is addressed by the toxicological studies made with the food enzyme.

Total peroxides (peracetic acid and hydrogen peroxide), used during the thermolabilisation step of food enzyme production process, were measured in three independent batches of the intermediate products before and after thermolabilisation, and in the same batches of the final food enzyme, and not detected in the latter ones.²¹

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. 10 mL of product were incubated in 90 mL of non-selective medium at 39°C for 60 h for resuscitation. From this, 10 × 1 mL were inoculated on selective agar plates and incubated at 39°C for 60 h. No colonies were produced. Appropriate positive controls were included.²²

¹⁷ Technical dossier/Annexes 1–3 and 12–14 and 27 and Additional data January 19/Annex 2.

¹⁸ LoDs: Pb = 5 mg/kg; As = 3 mg/kg; Cd = 0.5 mg/kg; Hg = 0.5 mg/kg.

¹⁹ Technical dossier/Annexes 12–14.

²⁰ Technical dossier/p. 33/Annex 15.

²¹ Technical dossier/Additional data December 2020/Annex Q4.

²² Technical dossier/Additional data December 2020/Annex Q3.

3.4. Toxicological data

A battery of toxicological tests, including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian chromosomal aberration test and a repeated dose 90-day oral toxicity study in rats, has been provided. The batch 4 (Table 1) used in these studies was 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 Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997a) and following Good Laboratory Practice (GLP).²³ Four strains of *Salmonella* Typhimurium (TA1535, TA1537, TA98 and TA100) and *Escherichia coli* WP2 *uvrA* were used in the presence or absence of metabolic activation (S9-mix), applying the treat and plate method. A single experiment was carried out in triplicate using eight concentrations of the food enzyme (from 1.5 to 5,000 µg/plate, corresponding to 0.08, 0.27, 0.80, 2.65, 7.95, 26.5, 79.5 and 265 µg TOS/plate). No cytotoxicity was observed at any concentration tested. Upon treatment with the food enzyme, there was no significant increase in revertant colony numbers above the control values in any strain with or without S9-mix.

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* mammalian chromosomal aberration test

The *in vitro* mammalian chromosomal aberration test was carried out in human peripheral blood lymphocytes according to OECD Test Guideline 473 (OECD, 1997b) and following GLP.²⁴ The food enzyme was tested in two separate experiments carried out in duplicate. Based on the results of a dose-finding study, in the first experiment, in the short-term treatment (3 h followed by 18 h recovery period), the cells were exposed to the food enzyme at 10, 1,000 and 2,520 µg/mL (corresponding to 0.53, 53 and 133.6 µg TOS/mL) with metabolic activation (S9-mix) and at 300, 1,000 and 2,520 µg/mL (corresponding to 15.9, 53 and 133.6 µg TOS/mL) without S9-mix. In the second experiment, the cells were exposed to the food enzyme at 100, 1,000 and 2,520 µg/mL (corresponding to 5.3, 53 and 133.6 µg TOS/mL) in the short-term treatment with S9-mix and at 100, 300, and 2,100 µg/mL (corresponding to 5.3, 15.9 and 111.3 µg TOS/mL) in the continuous 21-h treatment without S9-mix. The relative mitotic index at the highest concentration tested was 53% and 74% in the short-term treatments with S9-mix, 89% in the short-term treatment without S9-mix and 63% in the continuous treatment without S9-mix. The frequency of structural and numerical chromosomal aberrations in treated cultures was comparable to the values detected in negative controls and within the range of the laboratory historical control data.

The Panel concluded that food enzyme did not induce chromosome aberrations under the test conditions employed for this study.

3.4.2. Repeated dose 90-day oral toxicity study rodents

The repeated dose 90-day oral toxicity study was performed in accordance with OECD Test Guideline 408 (OECD, 1998) and following GLP.²⁵ Groups of 10 male and 10 female Charles River Han Wistar (CrI:WI(Han)) rats received 42, 140 and 420 mg enzyme proteins/kg bw per day of the food enzyme by gavage, corresponding to 62, 206 and 618 mg TOS/kg bw per day. Controls received the vehicle (sterile water).

No mortality was observed.

In the functional observations, statistically significant changes were observed for motor activity in some of six 10-min periods at the high dose. In high-dose males, movement counts and distance travelled were increased in period 1 (+96% and +79%, respectively) and in period 6 (+54% and 60%, respectively) and decreased in period 5 (−52% and −52%, respectively), while a time at rest was decreased in period 1 (−17%) and increased in period 5 (+18%). In high-dose females, movement counts were increased in period 1 (+67%) and decreased in period 3 (−27%), a distance travelled was increased in period 1 (+59%) and decreased in periods 3 (−25%) and 5 (−36%), while

²³ Technical dossier/Annex 24; Technical dossier/Spontaneous data submission, January 2019/Annex 1 and Annex 2.

²⁴ Technical dossier/Annex 25; Technical dossier/Spontaneous data submission, January 2019/Annex 1 and Annex 2.

²⁵ Technical dossier/Annex 26; Technical dossier/Spontaneous data submission, January 2019/Annex 1 and Annex 2.

a time at rest was decreased in period 1 (–27%) and increased in period 3 (+16%). The Panel considered these changes as not toxicologically relevant as they were only recorded sporadically and there was no consistency between males and females in the occurrence and the direction of a change for a given motor activity parameter in some of the measurement periods.

The haematological investigation revealed a statistically significant increase in haemoglobin concentration in low-dose females (+3.8%). The Panel considered the change as not toxicologically relevant as it was only observed in one sex, there was no dose–response relationship, the change was small and it was within the historical control values.

The clinical chemistry investigation revealed a statistically significant increase in total protein concentration (+3%, +1.6% and +3.4%) in all treated male groups, in cholesterol (+15%) in high-dose males, in glucose (+12% and +9.9%) in mid- and high-dose males, in alkaline phosphatase (ALP, +43.3%) in low-dose females and a decrease in calcium concentration (–3.8%) in high-dose females. 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 (glucose, ALP), the changes were small (total protein, ALP) and the changes were within the historical control values (all parameters).

Statistically significant changes in organ weights included a decrease in absolute spleen (–12%) and epididymides (–10%) weights in the high-dose males and in absolute testes weight in mid- and high-dose males (–8.8% and –8.8%, respectively). The Panel considered the changes as not toxicologically relevant as the changes were small (all parameters), they were only observed in one sex (spleen), there were no histopathological changes in the spleen, epididymides and testes and there were no correlating changes in the haematological parameters (spleen).

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

The Panel identified a no observed adverse effect level (NOAEL) of 618 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 mucorpepsin produced with the non-genetically modified *R. miehei* strain DSM 29547 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, three matches were found. The matching allergens were: P00791.3, pepsin A from *Sus scrofa*; CAA59419.1, an aspergillopepsin from *Aspergillus fumigatus*; ABU95411.1, a lipid transfer protein Sin a 3.01 from yellow mustard (*Sinapis alba*).

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

Pepsin from *Sus scrofa* as well as aspergillopepsin are associated with (occupational) asthma and rhinitis. However, several studies have shown that adults, sensitised to respiratory allergens may be able to ingest respiratory allergens without acquiring clinical symptoms of food allergy (Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). Mustard is a food allergen and listed in Annex II of the Regulation (EU) No 1169/2011²⁶. Sin a 3.01 is not a major allergen. Products that may cause allergies or intolerances (Regulation (EU) No 1169/2011²⁶) are used as raw materials () 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 foods employed as protein sources are not expected to be present.

The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme cannot be excluded, but is

²⁶ 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.

considered low except for individuals sensitised to mustard proteins, but this risk will not exceed that of mustard consumption.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in milk processing for cheese production at a recommended use level of 2–66 IMCU/kg milk, corresponding to 0.04–1.31 mg TOS/kg milk.²⁷

In cheese production, the food enzyme is added to the milk during the coagulation step to hydrolyze κ -casein. Whey is separated from the curd.²⁸ Curd is further processed into different types of cheese, whereas whey is used in the production of several foods, including bakery and beverages.

The food enzyme partitions in both curd and whey and remains in the final foods. The distribution between curd and whey is approximately 1:9 (Guinee and Wilkinson, 1992).

Based on thermostability data (see Section 3.3.1), the enzyme is not completely inactivated during pasteurisation of the whey.

3.5.2. Dietary exposure estimation

Chronic exposure to the food enzyme–TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSA CEP Panel, 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 2 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 to the food enzyme–TOS was estimated to be about 0.257 mg TOS/kg bw per day in infants.

Table 2: 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)	0.006–0.115 (11)	0.006–0.052 (15)	0.003–0.007 (19)	0.001–0.010 (21)	0.001–0.008 (22)	0.001–0.003 (22)
Min–max 95th percentile (number of surveys)	0.028–0.257 (9)	0.020–0.118 (13)	0.006–0.024 (19)	0.004–0.010 (20)	0.002–0.025 (22)	0.002–0.006 (21)

TOS: total organic solids.

3.5.3. Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the following sources of uncertainties have been considered and are summarised in Table 3.

²⁷ Technical dossier/p. 57.

²⁸ Technical dossier/p. 56.

Table 3: 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	+
Assuming that whey protein concentrate is used in all milk-based infant formulae and follow-on formulae	+
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	+/-

TOS: total organic solids.

+: Uncertainty with potential to cause overestimation of exposure.

-: Uncertainty with potential to cause underestimation of exposure.

The conservative approach applied to the exposure estimate to food enzyme-TOS, in particular assumptions made on the occurrence and use levels of this specific food enzyme, is likely to have led to overestimation of the exposure.

3.6. Margin of exposure

A comparison of the NOAEL (618 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0.001–0.115 mg TOS/kg bw per day at the mean and from 0.002 to 0.257 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposure (MoE) of at least 2,405.

4. Conclusions

Based on the data provided and the derived margin of exposure the Panel concluded that the food enzyme mucorpepsin produced with the *R. miehei* strain DSM 29547 does not give rise to safety concerns under the intended conditions of use.

5. Documentation as provided to EFSA

Application for authorisation of mucorpepsin from *Rhizomucor miehei* in accordance with Regulation (EC) No 1331/2008. March 2015. Submitted by Chr. Hansen. The dossier was updated on 23 January 2019.

Additional information. December 2020. Submitted by Chr. Hansen.

References

- Armentia A, Dias-Perales A, Castrodeza J, Dueñas-Laita A, Palacin A and Fernández S, 2009. Why can patients with baker's asthma tolerate wheat flour ingestion? Is wheat pollen allergy relevant? *Allergologia et Immunopathologia*, 37, 203–204.
- Brisman J, 2002. Baker's asthma. *Occupational and Environmental Medicine*, 59, 498–502.
- EFSA (European Food Safety Authority), 2006. Opinion of the Scientific Committee related to uncertainties in dietary exposure assessment. *EFSA Journal* 2006;5(1):438, 54 pp. <https://doi.org/10.2903/j.efsa.2007.438>
- EFSA (European Food Safety Authority), 2009a. Guidance of EFSA prepared by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. *EFSA Journal* 2009;7(8):1305, 26 pp. <https://doi.org/10.2903/j.efsa.2009.1305>
- EFSA (European Food Safety Authority), 2009b. Guidance of the Scientific Committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: general principles. *EFSA Journal* 2009;7(5):1051, 22 pp. <https://doi.org/10.2903/j.efsa.2009.1051>

- EFSA (European Food Safety Authority), 2011. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. *EFSA Journal* 2011;9(3):2097, 34 pp. <https://doi.org/10.2903/j.efsa.2011.2097>
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), 2019. Statement on the characterisation of microorganisms used for the production of food enzymes. *EFSA Journal* 2019;17(6):5741, 13 pp. <https://doi.org/10.2903/j.efsa.2019.5741>
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré C, Barat Baviera JM, Bolognesi C, Coconcelli 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, Gomes A, Kovalkovicova N, Liu Y, Rainieri S and Chesson A, 2021a. Scientific Guidance for the submission of dossiers on Food Enzymes. *EFSA Journal* 2021;19(10):6851, 37 pp. <https://doi.org/10.2903/j.efsa.2021.6851>
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré C, Barat Baviera JM, Bolognesi C, Coconcelli 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, Liu Y and Chesson A, 2021b. Statement on the process-specific technical data used in exposure assessment of food enzymes. *EFSA Journal* 2021;19(12):7010, 38 pp. <https://doi.org/10.2903/j.efsa.2021.7010>
- EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010. Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. *EFSA Journal* 2010;8(7):1700, 168 pp. <https://doi.org/10.2903/j.efsa.2010.1700>
- FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization), 2006. General specifications and considerations for enzyme preparations used in food processing in Compendium of food additive specifications. 67th meeting. FAO JECFA Monographs, 3, 63–67. Available online: <http://www.fao.org/3/a-a0675e.pdf>
- Guinee TP and Wilkinson MG, 1992. Rennet coagulation and coagulants in cheese manufacture. *Journal of the Society of Dairy Technology*, Vol 45, No 4 November 1992. <https://doi.org/10.1111/j.1471-0307.1992.tb01791.x>
- OECD (Organisation for Economic Co-Operation and Development), 1997a. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 471: bacterial reverse mutation test. 21 July 1997, 11 pp. Available online: <https://www.oecd-ilibrary.org/docserver/9789264071247-en.pdf?expires=1639400502&id=id&accname=guest&checksum=D2E89C16911BE1BEB13BBC206B402098>
- OECD (Organisation for Economic Co-Operation and Development), 1997b. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 473: in vitro mammalian chromosomal aberration test. 21 July 1997, 10 pp. Available online: https://www.oecd-ilibrary.org/environment/test-no-473-in-vitro-mammalian-chromosome-aberration-test_9789264071261-en
- OECD (Organisation for Economic Co-Operation and Development), 1998. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 408: repeated dose 90-day oral toxicity study in rodents. 21 September 1998, 10 pp. Available online: http://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en
- Poulsen LK, 2004. Allergy assessment of foods or ingredients derived from biotechnology, gene-modified organisms, or novel food. *Molecular Nutrition & Food Research*, 48, 413–423.

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
IMCU	International Milk Clotting Units
ITS	Internal transcribed spacer
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LC–DAD–TOFMS	liquid chromatography–diode–array detection–time–of–flight mass spectrometry
LoD	limit of detection
MoE	margin of exposure
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
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.2022.7457#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).