

ADOPTED: 8 July 2022

doi: 10.2903/j.efsa.2022.7462

Safety evaluation of the food enzyme chymosin from the genetically modified *Kluyveromyces lactis* strain CHY

EFSA Panel on Food Contact Materials, Enzyme and Processing Aids (CEP),
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, Jaime Aguilera, Magdalena Andryszkiewicz, Giulio Di Piazza,
Natalia Kovalkovicova, Yi Liu, Rita Ferreira de Sousa and Andrew Chesson

Abstract

The food enzyme chymosin (EC 3.4.23.4) is produced with the genetically modified *Kluyveromyces lactis* strain CHY by DSM Food Specialties B.V. It is intended to be used in milk processing for cheese production and for production of fermented milk products. Dietary exposure was estimated to be up to 0.69 mg total organic solids (TOS)/kg body weight (bw) per day in European populations. The production strain contains multiple copies of known antimicrobial resistance genes and consequently, it does not fully fulfil the requirements for the qualified presumption of safety (QPS) approach to safety assessment. However, considering the absence of viable cells and DNA from the production organism in the food enzyme, this is not considered to be a risk. As no other concerns arising from the microbial source and its subsequent genetic modification or from the manufacturing process have been identified, the Panel considered that toxicological tests were not needed for the assessment of this food enzyme. Similarity of the amino acid sequence of the food enzyme to those of known allergens was searched and four matches were found. The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions by dietary exposure, although unlikely, cannot be excluded, particularly for individuals sensitised to cedar pollen allergens. 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, chymosin, aspartic endopeptidases, rennin, EC 3.4.23.4, *Kluyveromyces lactis*, genetically modified microorganism

Requestor: European Commission

Question number: EFSA-Q-2015-00129

Correspondence: fip@efsa.europa.eu

Panel members: 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 and Andrew Chesson.

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

Acknowledgements: The Panel wishes to thank the following for the support provided to this scientific output: Ivana Nikodinoska and Simone Lunardi who made a contribution but are not eligible as authors. 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, Enzyme 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, Aguilera J, Andryszkiewicz M, Di Piazza G, Kovalkovicova N, Liu Y, de Sousa RF and Chesson A, 2022. Scientific Opinion on the safety evaluation of the food enzyme chymosin from the genetically modified *Kluyveromyces lactis* strain CHY. EFSA Journal 2022;20(8):7462, 15 pp. <https://doi.org/10.2903/j.efsa.2022.7462>

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/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
3.1.1. Characteristics of the parental and recipient microorganisms.....	6
3.1.2. Characteristics of introduced sequences.....	6
3.1.3. Description of the genetic modification process.....	6
3.1.4. Safety aspects of the genetic modification.....	7
3.2. Production of the food enzyme.....	7
3.3. Characteristics of the food enzyme.....	8
3.3.1. Properties of the food enzyme.....	8
3.3.2. Chemical parameters.....	8
3.3.3. Purity.....	8
3.3.4. Viable cells and DNA of the production strain.....	9
3.4. Toxicological data.....	9
3.4.1. 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.....	11
3.6. Margin of exposure.....	11
4. Conclusions.....	12
5. Documentation as provided to EFSA.....	12
References.....	12
Abbreviations.....	13
Appendix A – Dietary exposure estimates to the food enzyme–TOS in details.....	14
Appendix B – Population groups considered for the exposure assessment.....	15

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) list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7 (2) of Regulation (EC) No 1332/2008 on food enzymes.

Five applications have been introduced by the companies "Novozymes A/S", "DSM Food Specialties B.V.", "Advanced Enzyme Technologies Ltd" and the "Association of Manufacturing and Formulators of Enzyme Products (AMFEP)" for the authorisation of the food enzymes Pullulanase from a genetically modified strain of *Bacillus subtilis* (strain NZYM-AK), Glucoamylase from a genetically modified strain of *Aspergillus niger* (strain NZYM-BW), Chymosin from a genetically modified strain of *Kluyveromyces lactis* (strain CHY), Pectin lyase from a genetically modified strain of *Aspergillus niger* (FLOSC) and Triacylglycerol lipase from pregastric tissues of cattle, goat and sheep respectively.

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 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 assessment on the food enzymes Pullulanase from a genetically modified strain of *Bacillus subtilis* (strain NZYM-AK), Glucoamylase from a genetically modified strain of *Aspergillus niger* (strain NZYM-BW), Chymosin from a genetically modified strain of *Kluyveromyces lactis* (strain CHY), Pectin lyase from a genetically modified strain of *Aspergillus niger* (FLOSC) and Triacylglycerol lipase from pregastric tissues of cattle, goat and sheep 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 chymosin from a genetically modified *K. lactis* (strain CHY) from DSM Food Specialties B.V.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme chymosin from a genetically modified *K. lactis* (strain CHY).

Additional information was requested from the applicant during the assessment process on 7 December 2020 and subsequently 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 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	Chymosin
Systematic name	NA
Synonyms	Rennin
IUBMB No	EC 3.4.23.4
CAS No	9001-98-3
EINECS No	232-645-0

Chymosins catalyse the hydrolysis of a single peptide bond between amino acid residues 105 and 106, phenylalanine and methionine (Ser-Phe¹⁰⁵/Met¹⁰⁶-Ala) in κ -casein. This results in precipitation of milk protein and curd formation. The food enzyme is intended to be used in milk processing for cheese production and for the production of fermented milk products.

3.1. Source of the food enzyme

The chymosin is produced with the genetically modified *K. lactis* strain CHY [REDACTED], which is deposited in the Westerdijk Fungal Biodiversity Institute culture collection (CBS, The Netherlands) under number [REDACTED]⁴ The production strain was identified as *K. lactis* [REDACTED]

[REDACTED]⁵

⁴ Technical dossier/Additional information September 2021/Annex II-1.

⁵ Technical dossier/Additional information September 2021/Annex II-2.

The species *K. lactis* is included in the list of organisms for which the Qualified Presumption of Safety (QPS) may be applied (EFSA BIOHAZ Panel, 2022).

3.1.1. Characteristics of the parental and recipient microorganisms

6 [Redacted text]

3.1.2. Characteristics of introduced sequences

[Redacted text]

3.1.3. Description of the genetic modification process

The purpose of the genetic modification was to enable the production strain to synthesise prochymosin from *B. taurus*. [Redacted text]

[Redacted text]

⁶ Technical dossier/1st submission/p.97.

⁷ Technical dossier/Annex II-6.



3.1.4. Safety aspects of the genetic modification

The technical dossier contains all necessary information on the recipient microorganism, the donor organism and the genetic modification process.

The production strain contains multiple copies of the antimicrobial resistance genes [redacted]. Their presence in the production strain was considered a hazard, which is further discussed in this opinion.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004⁸, 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, 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, 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.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.

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/1st submission/p.50/Annex I-5.

¹⁰ Technical dossier/1st submission/pg. 50-58/Annex I-7.

¹¹ Technical dossier/1st submission/Annex I-7.

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The mature chymosin is a single polypeptide chain of 323 amino acids.¹² Chymosin is produced as prochymosin, and the pro-peptide is cleaved off as pH is reduced during downstream processing. The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be 36 kDa.¹³ 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 migrating between 36.5 and 55.4 kDa, consistent with the expected mass of the enzyme.¹³ No other enzyme activities were reported.¹⁴

The in-house determination of chymosin activity is based on the clotting of reconstituted skimmed milk (reaction conditions: pH 6.5, 37°C).¹⁵ The enzymatic activity is determined spectrophotometrically at 600 nm by measuring the time from the addition of the enzyme to an increase in optical density of 0.833. The chymosin activity is quantified relative to an enzyme standard and expressed in International Milk Clotting Unit/g (IMCU/g).¹⁶

The food enzyme has a temperature optimum around 37–45°C (pH 6.5) and a pH optimum around pH 5.8 (37°C). Thermostability was tested after a pre-incubation of the food enzyme for 10 min at different temperatures (pH 6.5). Enzyme activity decreased above 55°C, showing no residual activity above 65°C.¹⁶

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (Table 1).¹⁷ The mean total organic solids (TOS) was 9.9%. The mean enzyme activity/TOS ratio was 13.4 IMCU/mg TOS.

Table 1: Compositional data of the food enzyme

Parameter	Unit	Batch		
		1	2	3
Chymosin activity	IMCU/g batch ^(a)	1,300	1,390	1,270
Protein	%	2.5	3.1	2.9
Ash	%	10.2	1.5	1.3
Water	%	80.9	88.3	88.0
Total Organic Solids (TOS) ^(b)	%	8.9	10.2	10.7
Chymosin activity/mg TOS	IMCU/mg TOS	14.6	13.6	11.9

(a): IMCU: International Milk Clotting Unit (see Section 3.3.1).

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

3.3.3. Purity

The lead content in the three commercial batches was below 1 mg/kg^{18,19} 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).

The food enzyme complies with the microbiological criteria as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). No antimicrobial activity was detected in any of the tested batches (FAO/WHO, 2006).²⁰

¹² Technical dossier/1st submission/p. 41.

¹³ Technical dossier/1st submission/p. 39.

¹⁴ Technical dossier/1st submission/p. 40.

¹⁵ Technical dossier/1st submission/p. 44.

¹⁶ Technical dossier/Additional information September 2021/Annex 1.

¹⁷ Technical dossier/1st submission/p. 38 and 71/Annex I-3.

¹⁸ Technical dossier/1st submission/p. 72/ Annex I-3/Annex I-4.

¹⁹ LOD: Pb = 0.006 mg/l sample solution.

²⁰ Technical dossier/1st submission/p.72/Annex I-3/Annex I-4.

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]

²¹

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

²²

3.4. Toxicological data

Although all other requirements for the QPS have been met, the production strain carries copies of several antimicrobial resistance genes and therefore cannot be considered as suitable for the QPS approach. However, no risk is expected from the presence of these antimicrobial resistance genes, as the food enzyme has been shown not to contain viable cells and DNA of the production strain (Section 3.3.4). As no other concerns arising from the microbial source and its subsequent genetic modification or from the manufacturing process have been identified, the Panel considered that toxicological tests were not needed for the assessment of this food enzyme.

3.4.1. 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 chymosin produced with the genetically modified *K. lactis* strain CHY was assessed by comparing its amino acid sequence with those of known allergens according to the 'Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms' (EFSA GMO Panel, 2010). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, four matches were found.²³ The matching allergens were pepsin A from wild boar (*Sus scrofa*), aspartic protease-like protein Bla g2 from German cockroach (*Blattella germanica*), aspergillopepsin Asp f10 from the filamentous fungus *Aspergillus fumigatus* and protease CPA63 from Japanese cedar (*Cryptomeria japonica*).

Pepsin is a known respiratory allergen causing occupational asthma and rhinitis in cheese workers (Añíbarro Bausela and Fontela, 1996; Cartier et al., 1984; Marques et al., 2006). Aspergillopepsin, which is also commonly used in food industry, is involved in aspergillosis (Lee and Kolattukudy, 1995; Reichard et al., 1995). Japanese cedar protease has been described as a pollen allergen (Ibrahim et al., 2010), and Bla g2 protease from the German cockroach has also been described as a respiratory allergen (Arruda et al., 1995; Gustchina et al., 2005). None of these proteins were reported to be oral allergens. Individuals sensitised to respiratory allergens usually can ingest the allergens without developing allergic reactions (Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). Cedar pollen contain the respiratory allergen CPA63 (Ibrahim et al., 2010) and respiratory allergy to Cedar pollen is associated with the oral allergy syndrome (Midoro-Horiuti et al., 2003; Kiguchi et al., 2021). In this syndrome, allergic reactions are mainly in the mouth and seldomly lead to severe systemic anaphylaxis. However, oral allergy cannot be excluded after consumption.

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

According to the information provided, [REDACTED] that may cause allergies or intolerances (Regulation (EU) No 1169/2011²⁴), are used as raw materials. In addition, [REDACTED] known allergens, are also present in the media fed to the microorganisms.²⁵ However, during the fermentation process, these products will be degraded and

²¹ Technical dossier/Additional information September 2021/Annex II-4.

²² Technical dossier/Additional information September 2021/Annex II-3.

²³ Technical dossier/1st submission /p.75-76/Annex I-11.

²⁴ 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.

²⁵ Technical dossier/1st submission/Annex I-8.

utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the yeast 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, although unlikely, cannot be excluded, particularly for individuals sensitised to cedar pollen allergens.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in two 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 dosage of the food enzyme (mg TOS/kg RM) ^(b)
Milk processing for cheese production	Milk	2.1– 3.5
Milk processing to production of fermented milk product	Milk	0.07– 0.21

TOS: total organic solids.

(a): The name 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): Numbers in bold were used for calculation.

(c): Technical dossier/1st submission/p. 65.

In cheese production, the food enzyme is added to the milk together with the starter culture.²⁶ The addition of chymosin causes the milk to coagulate and to form curd. By separating the liquid whey from the solid curd, 80–90% of the added enzyme is found in the whey fraction and 10–20% is retained in the cheese (Documentation provided to EFSA N. 4), in which residual enzyme activity is expected. Whey produced during cheese making may be used in a variety of foods including infant and follow-on formula or food for special medical purposes.²⁷ The food enzyme–TOS remains in cheese and whey.

In the production of fermented milk products, such as yoghurt, the food enzyme is added to milk after pasteurisation.²⁸ Chymosin performs the same function as in cheese, making the viscosity of the fermented dairy products to increase.²⁹ The food enzyme–TOS remains in the fermented milk products, in which residual enzyme activity is expected.

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 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 mean and 95th percentile exposure to the food enzyme–TOS per age class, country and survey, as well as

²⁶ Technical dossier/p. 63.

²⁷ Additional data September 2021.

²⁸ Technical dossier/p. 64.

²⁹ Technical dossier/1st submission/p. 89.

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 0.687 mg TOS/kg bw per day in infants.

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)	0.016–0.309 (11)	0.017–0.139 (15)	0.008–0.019 (19)	0.004–0.026 (21)	0.002–0.022 (22)	0.002–0.007 (22)
Min–max 95th (number of surveys)	0.078–0.687 (9)	0.056–0.315 (13)	0.017–0.064 (19)	0.010–0.026 (20)	0.006–0.068 (22)	0.005–0.017 (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 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	+
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

Since toxicological tests are considered unnecessary by the Panel, a margin of exposure was not calculated.

4. Conclusions

Based on the data provided, the Panel concluded that the food enzyme chymosin produced with the genetically modified *K. lactis* strain CHY does not give rise to safety concerns under the intended conditions of use.

The production strain of the food enzyme contains multiple copies of known antimicrobial resistance genes. However, based on the absence of viable cells and DNA from the production organism in the food enzyme, this was not considered to be a risk.

5. Documentation as provided to EFSA

- 1) Dossier "Application for authorisation of chymosin from a genetically modified strain of *Kluyveromyces lactis* in accordance with Regulation (EC) No 1331/2008". January 2015. Submitted by DSM Food Specialties B.V.
- 2) Additional information. September 2021. Submitted by DSM Food Specialties B.V.
- 3) Summary report on technical data and dietary exposure. August 2016. Delivered by Hylobates Consulting (Rome, Italy) and BiCT (Lodi, Italy).
- 4) "Transfer of food enzymes into whey and cheese during dairy processing". January 2019. Provided by the Association of Manufacturers and Formulators of Enzyme Products.

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.
- Arruda LK, Vailes LD, Mann BJ, Shannon J, Fox JW, Vedvick TS, Hayden ML and Chapman MD, 1995. Molecular Cloning of a Major Cockroach (*Blattella germanica*) Allergen, Bla g 2. Molecular cloning of a major cockroach (*Blattella germanica*) allergen, Bla g 2. Sequence homology to the aspartic proteases.
- Bausela A and Fontela JL, 1996. Occupational asthma in a cheese worker. *Allergy*, 51, 960–961.
- van den Berg JA, van der Laken KJ, van Ooyen AJJ, Renniers TCHM, Rietveld K, Schaap A, Brake AJ, Bishop RJ, Schulz K, Moyer D, Richman M and Shuster JR, 1990. *Kluyveromyces* as a host for heterologous gene expression: expression and secretion of prochymosin. *Bio/Technology*, 8, 135–139.
- Brisman J, 2002. Baker's asthma. *Occupational and Environmental Medicine*, 59, 498–502.
- Cartier A, Malo J-L, Pineau L and Dolovich J, 1984. Occupational asthma due to pepsin. *Journal of Allergy and Clinical Immunology*, 73, 574–577.
- 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 BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Hilbert F, Lindqvist R, Nauta M, Peixe L, Ru G, Simmons M, Skandamis P, Suffredini E, Cocconcelli PS, Fernandez Escamez PS, Prieto-Maradona M, Querol A, Sijtsma L, Evaristo Suarez J, Sundh I, Vlaskovic J, Barizzone F, Hempen M and Herman L, 2022. Statement on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 15: suitability of taxonomic units notified to EFSA until September 2021. *EFSA Journal* 2022;20(1):7045, 40 pp. <https://doi.org/10.2903/j.efsa.2022.7045>
- 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, 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, 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>
- Gustchina A, Li M, Wünschmann S, Chapman MD, Pomés A and Wlodawer A, 2005. Crystal structure of cockroach allergen Bla g 2, an unusual zinc binding aspartic protease with a novel mode of self-inhibition. *Journal of Molecular Biology*, 348, 433–444.
- Ibrahim ARN, Kawamoto S, Aki T, Shimada Y, Rikimaru S, Onishi N, Babiker EE, Oiso I, Hashimoto K, Hayashi T and Ono K, 2010. Molecular cloning and immunochemical characterization of a novel major Japanese Cedar pollen allergen belonging to the aspartic protease family. *International Archives of Allergy Immunology*, 152, 207–218.
- Kiguchi T, Yamamoto-Hanada K, Saito-Abe M, Sato M, Irahara M and Ogita H, 2021. Pollen-food allergy syndrome and component sensitization in adolescents: a Japanese population-based study. *PLoS ONE*, 16, e0249649.
- Lee JD and Kolattukudy PE, 1995. Molecular cloning of the cDNA and gene for an elastinolytic aspartic proteinase from *Aspergillus fumigatus* and evidence of its secretion by the fungus during invasion of the host lung. *Infection and Immunity*, 63, 3796–3803.
- Marques L, Lara S, Abos T and Bartolome B, 2006. Occupational rhinitis due to Pepsin. *Journal of Investigation Allergology Clinical Immunology*, 16, 136–137.
- Midoro-Horiuti T, Mathura V, Schein CH, Braun W, Yu S, Watanabe M, Lee JC, Brooks EG and Goldblum RM, 2003. Major linear IgE epitopes of mountain cedar pollen allergen Jun a 1 map to the pectate lyase catalytic site. *Molecular Immunology*, 40, 555–562.
- 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.
- Reichard U, Monod M and Rüchel R, 1995. Molecular cloning and sequencing of the gene encoding an extracellular aspartic proteinase from *Aspergillus fumigatus*. *FEMS Microbiology Letters*, 130, 69–74.
- Sgorbati B, Scardovi V and Leblanc DJ, 1982. Plasmids in the genus *Bifidobacterium*. *Journal of Genetics Microbiology*, 128, 2121–2131.

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
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
QPS	qualified presumption of safety
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 <https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2022.7462#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).