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SCIENTIFIC OPINION

Safety evaluation of the food enzyme rennet containing chymosin and pepsin A from the abomasum of suckling calves, goats and lambs

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Abstract

The food enzyme rennet containing chymosin (EC 3.4.23.4) and pepsin A (EC 3.4.23.1) is prepared from the abomasum of suckling calves, goats and lambs by GENENCOR INTERNATIONAL B.V. The food enzyme is intended to be used in milk processing for cheese production. As no concerns arise from the animal source of the food enzyme or from its manufacture and based on the history of safe use and consumption, the Panel considered that toxicological data and the estimation of dietary exposure were not required. On the basis of literature data, the Panel considered that the risk of allergic reactions by dietary exposure could not 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

abomasum, chymosin, EC 3.4.23.1, EC 3.4.23.4, food enzyme, pepsin A, suckling calves, suckling goats, suckling lambs

CONTENTS

1 | INTRODUCTION

Article 3 of the Regulation (EC) No 1332/2008[1] provides definition for 'food enzyme' and 'food enzyme preparation'.

'Food enzyme' means a product obtained from plants, animals or microorganisms or products thereof including a product obtained by a fermentation process using microorganisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.

'Food enzyme preparation' means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

Before January 2009, food enzymes other than those used as food additives were not regulated or were regulated as processing aids under the legislation of the Member States. On 20 January 2009, Regulation (EC) No 1332/2008[1] 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 EU 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 CEF Panel, 2009) 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[1] on food enzymes.

Six applications have been introduced by the companies "Decernis, LLC", "Keller and Heckman LLP", the Association of Manufacturers and Formulators of Enzyme Products (AMFEP)" and "Novozymes A/S" for the authorisation of food enzymes cyclomaltodextrin glucanotransferase from *Geobacillus stearothermophilus*, dextranase from *Chaetomium gracile*, subtilisin from *Bacillus licheniformis*, mucorpepsin from *Rhizomucor miehei*, animal rennet consisting of chymosin and pepsin from the abomasum of *Bos primigenius* (cattle), *Bubalus bubalis* (buffalo), *Capra aegagrus hircus* (goat) and *Ovis aries* (sheep), and lipase from a genetically modified strain of *Aspergillus niger* (strain NZYM-DB) respectively.

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008,[2] 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.

1.1.2 | Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes cyclomaltodextrin glucanotransferase from *Geobacillus stearothermophilus*, dextranase from *Chaetomium gracile*, subtilisin from *Bacillus licheniformis*, mucorpepsin from *Rhizomucor miehei*, animal rennet consisting of chymosin and pepsin from the abomasum of *Bos primigenius* (cattle), *Bubalus bubalis* (buffalo), *Capra aegagrus hircus* (goat) and *Ovis aries* (sheep), and lipase from a genetically modified strain of *Aspergillus niger* (strain NZYM-DB) in accordance with Article 17.3 of Regulation (EC) No 1332/2008[1] on food enzymes.

[1]Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on Food Enzymes and Amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L 354, 31.12.2008, pp. 7–15.

[2]Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, pp. 1–6.

[3]Commission Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 64, 11.3.2011, pp. 15–24.

1.2 | Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of food enzyme animal rennet consisting of chymosin and pepsin from the abomasum of *Bos primigenius* (cattle), *Bubalus bubalis* (buffalo), *Capra aegagrus hircus* (goat) and *Ovis aries* (sheep).

The application was submitted initially as a joint dossier[4] and identified as the EFSA-Q-2015-00237. During the risk assessment phase, it was found that the technical dossier is too generic to be evaluated. A solution was found on 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 joint dossier will be split into 10 individual data packages.

The current opinion addresses one data package originating from the joint dossier EFSA-Q-2015-00237. This data package, identified as EFSA-Q-2022-00585, concerns the food enzyme animal rennet consisting of chymosin and pepsin from the abomasum of *B. primigenius* (cattle), *B. bubalis* (buffalo), *C. aegagrus hircus* (goat) and *O. aries* (sheep) submitted by GENENCOR INTERNATIONAL B.V.

As the current accepted scientific name of cattle is *Bos taurus*, this name will be used in this opinion instead of *B. primigenius*.[6]

In the Additional information received on 26 June 2023 the applicant clarified that he obtains rennet from the abomasum of suckling calves (*B. taurus*), suckling goats (*C. aegagrus hircus*) and blend rennet from the abomasum of suckling calves (*B. taurus*) and suckling lambs (*O. aries*). The abomasum of suckling buffalo calves *(Bubalus bubalis*) is not used as raw material.[7] Therefore, the Panel did not perform a safety evaluation of the food enzyme rennet containing chymosin and pepsin A from the abomasum of suckling buffalo calves.

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme 'Animal rennet from *B. taurus* (cattle), *C. aegagrus hircus* (goat) and *O. aries* (sheep)'. The data package was submitted on 21 September 2022.

Additional information was requested from the applicant during the assessment process on 27 March 2023 and received on 26 June 2023 (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, 2009) and following the relevant guidance documents of the EFSA Scientific Committee.

The 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA CEF Panel, 2009) has 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, 2021).

3 | ASSESSMENT[8]

The food enzyme under application contains two declared activities: chymosin and pepsin A.

IUBMB nomenclature	Chymosin
Synonyms	Rennin, preprorennin
IUBMB No	3.4.23.4
CAS No	9001-98-3
EINECS No	232-645-0

[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 relevance. OJ L 168, 28.6.2012, p. 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

[6]Technical dossier/Additional information, 26 June 2023/Annex 1-Rennet EFSA-Q-2022-00585.

[7]Technical dossier/Additional information, 26 June 2023/Annex 1-Rennet EFSA-Q-2022-00585.

[8]Technical dossier/p. 4, 23, 29, 61.

Chymosin is an aspartic endopeptidase that catalyses the hydrolysis of the 104-Ser-Phe / Met-Ala-107 bonds of κ-casein, resulting in the destabilisation of casein micelles and causing milk to clot.

IUBMB nomenclature	Pepsin A
Synonyms	Pepsin; lactated pepsin; pepsin fortior; fundus-pepsin
IUBMB No	3.4.23.1
CAS No	9001-75-6
EINECS No	232-629-3

Pepsin A, an aspartic endopeptidase, hydrolyses peptide bonds in proteins and peptide molecules with the formation of shorter peptides and free amino acids. It preferentially cleaves peptide bonds between hydrophobic, preferably aromatic, amino acids.

The food enzyme is intended to be used in milk processing for cheese production.

3.1 | Source of the food enzyme[9]

The food enzyme rennet is prepared from the abomasum of suckling calves (*B. taurus*), goats (*C. aegagrus hircus*) and lambs (*O. aries*), fit for human consumption.[10]

The abomasa come from certified slaughterhouses,[11] surveyed and approved by the competent authorities. The food enzyme is exclusively obtained from healthy animals slaughtered under the supervision of official health authorities, following the requirements of the relevant EU hygiene regulations, the Food Hygiene Regulation (EC) No 852/2004[12] and Regulation (EC) No 853/2004.[13] Examples of certificates from slaughterhouses were provided by the applicant, confirming that animal tissues used for the preparation of the food enzyme comply with meat inspection requirements and are handled in accordance with good hygiene practice.[14]

In the EU, according to Regulation (EC) 1774/2002,[15] the abomasum of goat, sheep and cattle is considered fit for human consumption. It is an edible offal as defined in Regulation (EC) No 853/2004.[16]

No issues of concern arising from the safety of the source material were identified by the Panel.

3.2 | Production of the food enzyme[17]

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004,[18] with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[19]

The food enzyme is extracted from the abomasum of suckling calves, goats and lambs. After the animals are slaughtered, abomasa are emptied and then frozen before processing. The frozen abomasa are minced and extracted with [redacted]. The enzyme solution is separated from tissue residues with a filter press containing a textile pipe filter. The solution containing the food enzyme is then activated by [redacted], then neutralised, clarified, filtered and concentrated (e.g. by ultra-filtration). The concentrated food enzyme is formulated with [redacted]. The food enzyme may also be dried to powder or granulated.[20]

[9]Technical dossier/p. 5, 32–33; Technical dossier/Annex J.

[10]Technical dossier/p. 13; Technical dossier/Annex J.

[11]Technical dossier/Additional information, 26 June 2023/Annex 1-Rennet EFSA-Q-2022-00585.

[12]Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 139, 30.4.2004, pp. 54.

[13]Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. OJ L226, p. 22, 25.6.2004.

[14]Technical dossier/p. 32; Technical dossier/Additional information, 26 June 2023/Annex 1-Rennet EFSA-Q-2022-00585 and Annex N_SI_Certificates compliance meat inspection requirements.

[15]Regulation (EC) No 1774/2002 of the European Parliament and of the Council of 3 October 2002 laying down health rules concerning animal by-products not intended for human consumption. OJ L 273, 10.10.2002, p. 209.

[16]Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. OJ L226, 25.6.2004, p. 22.

[17]Technical dossier/p. 5–6, 13, 35–39; Technical dossier/Annex G; Annex H; Annex I; Annex K.

[18]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.

[19]Technical dossier/p. 5–6, 35; Technical dossier/Annex G; Annex H; Technical dossier/Additional information, 26 June 2023/Annex S_SI_French sanitaire agreement.

[20]Technical dossier/p. 13, 35–39.

Rennet obtained from the abomasum of suckling calves, goats and lambs is then used in three types of products: (1) [redacted] (2) [redacted] and (3) [redacted].[21]

The applicant provided information on the identity of the substances used in the extraction and in the subsequent downstream processing.[22]

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

Data from the literature indicate that the chymosin from the abomasum of suckling calves, goats and lambs is a single polypeptide chain of 381 amino acids[23] with a molecular mass of about 36.5 kDa (Kumar et al., 2010).[24]

The pepsin A from the abomasum of suckling calves, goats and lambs is a single polypeptide chain of 386 amino acids[25] with a molecular mass of 35 kDa of the mature protein (Munoz et al., 2004).[26]

No other enzyme activities were reported by the applicant.[27]

The determination of chymosin and pepsin activities is based on the official method ISO11815|IDF157, 2007.[28] The time needed for visual flocculation of a standard milk substrate prepared with a calcium chloride solution of 0.5 g per litre (pH ≈ 6.5) is determined. The clotting time of a rennet sample is compared under identical chemical and physical conditions to that of a reference standard with known milk-clotting activity and having the same enzyme composition as the sample, determined using the International Dairy Federation (IDF) standard 110 (IDF110B). The total milk-clotting activity is expressed in International Milk-Clotting Units (IMCU).

The relative contents of chymosin and pepsin A present in the calf rennet were determined by chromatographic analysis, based on the recognised official method ISO 15163|IDF 110, 2012 for milk and milk products, for calf rennet and adult bovine rennet.[29]

The food enzyme rennet from the abomasum of suckling calves has a temperature optimum around 45–50°C (pH 6.4).[30]

Data from literature indicate that the pH optimum for calf chymosin is around pH 4 and for calf pepsin is around pH 2.[31] A similar temperature/pH profile was determined for lamb rennet (Bouras & Aïssaoui-Zitoun, 2022; Rogelj et al., 2001)[32] and goat rennet (Moschopoulou, 2011; Moschopoulou et al., 2006).

3.3.2 | Chemical parameters[33]

Data on the chemical parameters of the food enzyme were provided for nine batches used for commercialisation, three from the abomasa of calves (Table 1), three from the abomasa of goats (Table 2) and three from abomasa of calves and lambs ([redacted] Table 3). The mean total organic solids (TOS) were 2.29%, 1.69% and 2.28%, respectively, and the mean enzyme activity/TOS ratio was 11.11, 3.5 and 6.49 IMCU/mg TOS, respectively.

TABLE 1 Composition of the food enzyme rennet from the abomasum of suckling calves ([redacted]).[c]

Parameters	Unit	Batch 1	Batch 2	Batch 3
Enzyme activity	IMCU/g[a]	256	256	250
Protein	%	1.8	1.9	2.0
Ash	%	17.88	17.9	17.86

[21]Technical dossier/Additional information, 26 June 2023/Annex 1-Rennet EFSA-Q-2022-00585.

[22]Technical dossier/Annex K.

[23]https://www.ncbi.nlm.nih.gov/protein/NP_001272688.1; https://www.ncbi.nlm.nih.gov/protein/NP_001009804.1; https://www.ncbi.nlm.nih.gov/protein/NP_851337.1

[24]Technical dossier/p. 29; Brenda Database.

[25]https://www.ncbi.nlm.nih.gov/protein/XP_005699798.1; https://www.ncbi.nlm.nih.gov/protein/XP_004019629.2; https://www.ncbi.nlm.nih.gov/protein/NP_001001600.2

[26]Technical dossier/p. 29; Brenda Database.

[27]Technical dossier/p. 4, 28.

[28]Technical dossier/p. 27; Technical dossier/Annex D; Technical dossier/Additional information, 26 June 2023/Annex 1-Rennet EFSA-Q-2022-00585.

[29]Technical dossier/p. 28; Technical dossier/Annex E.

[30]Technical dossier/p. 31.

[31]Technical dossier/p. 30; Brenda Database.

[32]Technical dossier/Additional information, 26 June 2023/Annex 1-Rennet EFSA-Q-2022-00585; Technical dossier/Additional information, 26 June 2023/Annex T; Annex U.

[33]Technical dossier/p. 26–27; Technical dossier/Annex A; Annex C; Technical dossier/Additional information, 26 June 2023/Annex 1-Rennet EFSA-Q-2022-00585/Annex P.

TABLE 1 (Continued)

Parameters	Unit	Batch 1	2	3
Water	%	79.9	79.8	79.8
Total organic solids (TOS)[b]	%	2.22	2.30	2.34
Activity/mg TOS	IMCU/mg TOS	11.53	11.13	10.68

[a]IMCU: International Milk-Clotting Units (see Section 3.3.1).

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

[c]Technical dossier/Additional information, 26 June 2023/Annex 1-Rennet EFSA-Q-2022-00585; Technical dossier/Additional information, 26 June 2023/Annex R; Annex O; Annex P; Annex Q.

TABLE 2 Composition of the food enzyme rennet from the abomasum of suckling goats ([redacted]).[c]

Parameters	Unit	Batch 1	2	3
Enzyme activity	IMCU/mL[a]	59	59	59
Protein	%	1.5	1.3	1.4
Ash	%	18.59	18.57	18.72
Water	%	79.8	79.7	79.5
Total organic solids (TOS)[b]	%	1.61	1.73	1.78
Activity/mg TOS	IMCU/mg TOS	3.66	3.41	3.43

[a]IMCU: International Milk-Clotting Units (see Section 3.3.1).

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

[c]Technical dossier/Additional information, 26 June 2023/Annex 1-Rennet EFSA-Q-2022-00585; Technical dossier/Additional information, 26 June 2023/Annex R; Annex O; Annex P; Annex Q.

TABLE 3 Composition of the food enzyme rennet blend from the abomasum of suckling calves and lambs ([redacted]).[c]

Parameters	Unit	Batch 1	2	3
Enzyme activity	IMCU/g[a]	148	148	148
Protein	%	1.9	1.9	2.0
Ash	%	18.24	18.20	18.22
Water	%	79.5	79.5	79.5
Total organic solids (TOS)[b]	%	2.26	2.30	2.28
Activity/mg TOS	IMCU/mg TOS	6.55	6.43	6.49

[a]IMCU: International Milk-Clotting Units (see Section 3.3.1).

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

[c]Technical dossier/Additional information, 26 June 2023/Annex 1-Rennet EFSA-Q-2022-00585; Technical dossier/Additional information, 26 June 2023/Annex R; Annex O; Annex P; Annex Q.

3.3.3 | Purity[34]

The lead content[35] in the three commercial batches of [redacted], [redacted] rennet was below 5 mg/kg, which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).

The food enzyme preparation of [redacted] rennet complies with the microbiological criteria for total coliforms, *Escherichia coli* and *Salmonella*, as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[36] *Listeria monocytogenes* was not detected in 25 g samples of the food enzyme batches.[37]

[34]Technical dossier/p. 4, 28, 61; Technical dossier/Annex A; Annex B; Annex C; Technical dossier/Additional information, 26 June 2023/Annex R; Annex O; Annex P; Annex Q.

[35]Technical dossier/Additional information, 26 June 2023/Annex R; Technical dossier/Additional information, 26 June 2023/Annex P: Limit of quantification (LoQ) for lead = 0.01 mg/kg.

[36]Technical dossier/p. 4, 28, 61; Technical dossier/Annex B; Annex C; Technical dossier/Additional information, 26 June 2023/Annex O; Annex Q.

[37]Technical dossier/Additional information, 26 June 2023/Annex O; Annex Q.

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

3.4 | Toxicological data[38]

According to the Commission Implementing Regulation (EU) No 562/2012[39], an application for the safety evaluation of a food enzyme does not need to include toxicological data if the food enzyme is obtained from edible parts of animals intended or reasonably expected to be ingested by humans.

According to the 'EFSA Guidance on the submission of a dossier on food enzymes for safety evaluation', the justification for not supplying toxicological data may include a documented history on the safety of the source of the food enzyme, the composition and the properties of the food enzyme, as well as its use in foods, demonstrating no adverse effects on human health when consumed in a comparable way (EFSA CEF Panel, 2009).

The Panel considered that these requirements are fulfilled, because:

(i) Rennet obtained from the abomasum of suckling calves, goats and lambs has been safely used in the production of cheese and related products for many centuries;
(ii) The abomasum from suckling calves, goats and lambs is consumed throughout the EU and elsewhere in the world as a meat product;[40]
(iii) The manufacturing process of the food enzyme is not considered to introduce substances that could raise safety concerns;
(iv) The compositional and purity data provided on the food enzyme are considered sufficient.

The Panel considered that sufficient information has been provided on the animal source, its history of safe use and consumption, and the manufacturing process. Therefore, the need for toxicological data was waived.

3.4.1 | Allergenicity[41]

The comparison of the amino acid sequences of chymosin and pepsin A with those of known allergens was considered unnecessary because their allergenic potential is already known.

Occupational respiratory allergies and skin sensitisation to dust of chymosin and pepsin, including from the animal sources of the food enzyme, have been described in workers upon industrial exposure and in medical laboratory technicians (Cartier et al., 1984; Gómez Torrijos et al., 2018; Jensen et al., 2006; Khan & Selamoglu, 2020; van Kampen et al., 2013). However, several studies have shown that adults with occupational asthma to an enzyme can commonly ingest the corresponding respiratory allergens without acquiring clinical symptoms of food allergy (Armentia et al., 2009; Brisman, 2002; Cullinan et al., 1997; Poulsen, 2004). There are no reports in the literature on adverse reactions upon ingestion of these enzymes in individuals sensitised through the respiratory route.

The Panel considered that allergic reactions to this food enzyme obtained from the abomasum of suckling calves, goats and lambs 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 milk processing for cheese production[42] at the recommended use level from around 1 to 16 mg TOS/kg milk.[43]

Animal rennet is added to milk to separate milk into solid curd and liquid whey (coagulation). Both chymosin and pepsin A contribute to the milk-clotting activity.[44] The majority of the food enzyme–TOS partitions into the whey and is mostly removed during the draining of the whey. Only a small portion of the food enzyme–TOS remains in the curd (ca. 6%–12%). The remaining rennet contributes to the ripening of cheese due to its general proteolytic activity.[45]

Based on data provided on thermostability (see Section 3.3.1), it was expected that the food enzyme may remain active in cheese, depending on the cheese-making process.

[38]Technical dossier/p. 8, 13, 32, 50–51.
[39]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. OJ L 168, 28.6.2012, p. 21–23.
[40]Technical dossier/p. 8.
[41]Technical dossier/p. 9, 51–54; Technical dossier/Annex M.
[42]Technical dossier/Additional information, 26 June 2023/Response 4.1.
[43]Technical dossier/p. 44, Technical dossier/Additional information, 26 June 2023/Responses 4.2 and 4.3.
[44]Technical dossier/p. 42–43.
[45]Technical dossier/p. 41–46.

3.5.2 | Dietary exposure estimation

The technology of extracting enzymes from animal abomasum and the technology of using animal rennet for cheese making have remained the same over thousands of years and remains the major source of human exposure to the food enzyme. Cheese and by-products of cheese making have been consumed by humans in Europe and many other parts of the world for millennia. In addition, the abomasum from ruminants is consumed in some European countries, although this constitutes only a minor fraction of the overall exposure to the food enzyme in EU. Accordingly, in the view of the Panel, a dietary exposure estimation was not required.

3.6 | Margin of exposure

Since the Panel considered that a toxicological assessment was unnecessary and the estimation of a dietary exposure not required, the margin of exposure was not calculated.

4 | CONCLUSION

Based on the data provided, the origin of the food enzyme and its history of safe use, the Panel concluded that the food enzyme rennet containing chymosin and pepsin A obtained from the abomasum of suckling calves, goats and lambs does not give rise to safety concerns under the intended conditions of use.

5 | DOCUMENTATION AS PROVIDED TO EFSA

Technical dossier 'Animal rennet from *Bos primigenius* (cattle), *Bubalus bubalis* (buffalo), *Capra aegagrus hircus* (goat) and *Ovis aries* (sheep)'. 16 January 2015 (joint dossier). The data package was submitted by GENENCOR INTERNATIONAL B.V. on 21 September 2022.

Technical dossier Additional information. 26 June 2023. Submitted by GENENCOR INTERNATIONAL B.V.

ABBREVIATIONS

AMFEP	Association of Manufacturers and Formulators of Enzyme Products
CAS	Chemical Abstracts Service
EFSA CEF Panel	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
EFSA CEP Panel	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
IDF	International Dairy Federation
IMCU	International Milk-Clotting Unit
ISO	International Organization for Standardization
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LOQ	limit of quantification
TOS	total organic solids
WHO	World Health Organization

CONFLICT 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.

REQUESTOR

European Commission

QUESTION NUMBER

EFSA-Q-2022-00585

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NOTE

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

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