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## Safety evaluation of the food enzyme rennet paste from the abomasum of suckling goats, lambs and calves

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### Abstract

The food enzyme rennet paste containing chymosin (EC 3.4.23.4), pepsin A (EC 3.4.23.1) and triacylglycerol lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) is prepared from the abomasum of suckling goats, lambs and calves by Caglificio Clerici S.p.A. 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, from its manufacture, and based on the history of safe use and consumption, the Panel considers that toxicological data were not required and no exposure assessment was necessary. On the basis of literature data, the Panel considers that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions by dietary exposure could not be excluded, but the likelihood is considered to be low. Based on the data provided, the Panel concludes that this food enzyme does not give rise to safety concerns under the intended conditions of use.

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**Keywords:** food enzyme, chymosin, EC 3.4.23.4, pepsin A, EC 3.4.23.1, triacylglycerol lipase, EC 3.1.1.3, abomasum, suckling goats, suckling lambs, suckling calves

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† Deceased.

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## 1. Introduction

Article 3 of the Regulation (EC) No 1332/2008<sup>1</sup> 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<sup>1</sup> 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<sup>2</sup> 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 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<sup>1</sup> on food enzymes.

Five applications have been introduced by the companies 'Amano Enzyme Inc.' for the authorisation of the food enzymes pullulanase from *Klebsiella pneumoniae* (strain AE-PUL), pullulanase from *Pullulanibacillus naganoensis* (strain AE-PL), and rhizopuspepsin from *Rhizopus niveus* (strain AE-N), 'Caglificio Clerici S.p.A.' for the authorisation of the food enzyme rennet paste from abomasum of goat (*Capra aegagrus hircus*), sheep (*Ovis aries*) and cattle (*Bos primigenius*), and 'Danisco US Inc.' for the authorisation of the food enzyme cellulase from a genetically modified strain of *Trichoderma reesei* (DP-Nzc36).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011<sup>3</sup> implementing Regulation (EC) No 1331/2008<sup>2</sup>, the Commission has verified that the application falls within the scope of the food enzyme Regulation and contains all the elements required under Chapter II of that Regulation.

#### 1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes pullulanase from *Klebsiella pneumoniae* (strain AE-PUL), pullulanase

<sup>1</sup> 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.

<sup>2</sup> 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.

<sup>3</sup> 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, p. 15–24.

from *Pullulanibacillus naganoensis* (strain AE-PL), rhizopuspepsin from *Rhizopus niveus* (strain AE-N), rennet paste from abomasum of goat (*Capra aegagrus hircus*), sheep (*Ovis aries*) and cattle (*Bos primigenius*), and cellulase from a genetically modified strain of *Trichoderma reesei* (DP-Nzc36) in accordance with Article 17.3 of Regulation (EC) No 1332/2008<sup>1</sup> 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 rennet paste from the abomasum of suckling goats (*Capra aegagrus hircus*), lambs (*Ovis aries*) and calves (*Bos primigenius*).

## 2. Data and methodologies

### 2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme rennet paste from abomasum of suckling goats (*C. aegagrus hircus*), lambs (*O. aries*) and calves (*B. primigenius*).

Additional information was requested from the applicant during the assessment process on 3 June 2021 and received on 1 September 2021 (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 existing guidance documents of EFSA Scientific Committee.

The current '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 to the methodology described in the CEF Panel 'Statement on the exposure assessment of food enzymes' (EFSA CEF Panel, 2016).

## 3. Assessment<sup>4</sup>

The food enzyme under application contains three declared activities:

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

Chymosin catalyses the hydrolysis of a single peptide bond between amino acid residues 105 and 106, phenylalanine and methionine (Ser-Phe105/Met-Ala) of  $\kappa$ -casein. This results in precipitation of milk protein and curd formation.

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

<sup>4</sup> Technical dossier/p. 4-9, 17, 21, 33-36, 55-56, 79-84.

IUBMB nomenclature	Triacylglycerol lipase
Systematic name	Triacylglycerol acylhydrolase
Synonyms	Lipase, triglyceride lipase; tributyrase; butyrinase; glycerol ester hydrolase
IUBMB No	EC 3.1.1.3
CAS No	9001-62-1
EINECS No	232-619-9

Triacylglycerol lipase catalyses the hydrolysis of triacylglycerols into fatty acids, mono- and diacylglycerols. It preferably hydrolyses ester bonds at positions one and three of the triacylglycerol molecule. However, triacylglycerol lipase hydrolyses also ester bonds of other lipids, including phospholipids and glycolipids.

The food enzyme is intended to be used in milk processing for cheese production.<sup>5</sup>

### 3.1. Source of the food enzyme<sup>6</sup>

The food enzyme rennet paste is prepared from the abomasum of suckling goats (*C. aegagrus hircus*), lambs (*O. aries*) or calves (*B. taurus*), fit for human consumption.<sup>7</sup> The abomasa retain their content of clotted milk, in order to preserve the enzymatic complex typical of rennet paste.<sup>8</sup>

The abomasa come from certified European and non-European slaughterhouses,<sup>9</sup> 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<sup>10</sup> and Regulation (EC) No 853/2004<sup>11</sup>. 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 hygienic practice.<sup>12</sup>

In the EU, according to Regulation (EC) 1774/2002<sup>13</sup>, 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.<sup>11</sup>

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

### 3.2. Production of the food enzyme<sup>14</sup>

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004<sup>10</sup>, with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.<sup>15</sup>

The food enzyme is extracted from the abomasum of suckling goats, lambs and calves.

The applicant provided the method of production of three rennet paste products, described as 'traditional', 'soluble' and 'reconstituted'.<sup>16</sup>

In all three rennet paste products, abomasa are prepared before their use for the production of rennet paste. They are trimmed to remove other tissues, but still contain clotted milk, preserved with sodium chloride, and then stored in a cool place before processing.

<sup>5</sup> Technical dossier/p. 6, 9, 81.

<sup>6</sup> Technical dossier/p. 5–6, 11; 45, 79; Technical dossier/Additional information, 1 September 2021.

<sup>7</sup> Technical dossier/p. 7.

<sup>8</sup> Technical dossier/p. 11, 19, 33.

<sup>9</sup> Technical dossier/p. 51.

<sup>10</sup> 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.

<sup>11</sup> 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.

<sup>12</sup> Technical dossier/Additional information, 1 September 2021.

<sup>13</sup> 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, p. 209, 10.10.2002.

<sup>14</sup> Technical dossier/p. 11–12, 19, 33, 45–54; Technical dossier/Annex 13; Technical dossier/Additional information, 1 September 2021.

<sup>15</sup> Technical dossier/p. 11, 47; Technical dossier/Annex 15.

<sup>16</sup> Technical dossier/p. 11, 19.

In the production of the 'traditional' and 'soluble' rennet pastes, abomasa are ground with sodium chloride and mixed to obtain a homogeneous product. In case of 'soluble' rennet paste, the ground abomasa are mixed with water and passed through a series of sieves to remove potential residues of other tissues. The obtained food enzyme concentrates are monitored for their specific activities and further standardised with sodium chloride and milk protein.

In the production of 'reconstituted' rennet paste, liquid extracts of abomasa are mixed with sodium benzoate and sodium chloride to obtain a food enzyme concentrate that is monitored for its specific activities and further standardised with sodium chloride.

Traditionally, some types of rennet paste are obtained from a single animal species, other types are obtained by mixing two or three different species of animals.<sup>12</sup>

The applicant provided information on the identity of the substances used in the extraction and in the subsequent downstream processing of the food enzyme.<sup>17</sup>

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<sup>18</sup>

The chymosin from the abomasum of suckling goats, lambs and calves is a single polypeptide chain of 381 amino acids.<sup>19</sup> The molecular mass of the mature protein was calculated to be 36.5 kDa (Kumar et al., 2010).<sup>20</sup>

The pepsin A from the abomasum of suckling goats, lambs and calves is a single polypeptide chain of 386 amino acids.<sup>21</sup> The molecular mass of the mature protein was calculated to be 35 kDa (Munoz et al., 2004).<sup>20</sup>

The triacylglycerol lipase from the abomasum of suckling goats, lambs and calves is a single polypeptide chain of 397 amino acids.<sup>22</sup> The molecular mass of the mature protein was calculated to be 45.2 kDa.<sup>23</sup>

No other enzymatic activities were reported by the applicant.<sup>24</sup>

The food enzyme was analysed by HPLC. The chromatogram of an unidentified paste rennet showed several major peaks, accompanied by some minor peaks.<sup>25</sup>

The determination of chymosin and pepsin activities is based on the official method ISO 11815|International Dairy Federation (IDF) standard 157:2007. The time needed for visual flocculation of a standard milk substrate prepared with a calcium chloride solution of 0.5 g per litre (pH  $\approx$  6.5) is determined.<sup>26</sup> 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 ISO 15163|IDF standard 110:2012.<sup>27</sup> The total milk-clotting activity is expressed in International Milk-Clotting Units (IMCU).

The triacylglycerol lipase activity of the rennet paste is determined according to the official method ISO 13082|IDF standard 218:2011 that is based on the hydrolysis of tributyrin (reaction conditions: pH 6.2, 42°C). The enzymatic activity is determined by back titration of the excess of sodium hydroxide used to release butyric acid from tributyrin. The amount of sodium hydroxide consumed within a defined period is used to calculate the activity in International Lipase Units (ILU) per millilitre or ILU per gram. One ILU is defined as the amount of lipase activity that releases butyric acid at a rate of 1.25  $\mu$ mol/min under the specified conditions.<sup>28</sup>

<sup>17</sup> Technical dossier/Annex 13.

<sup>18</sup> Technical dossier/p. 39–40; Technical dossier/Additional information, 1 September 2021.

<sup>19</sup> Technical dossier/Additional information, 1 September 2021/ [https://www.ncbi.nlm.nih.gov/protein/NP\\_001272688.1](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_001009804.1); [https://www.ncbi.nlm.nih.gov/protein/NP\\_851337.1](https://www.ncbi.nlm.nih.gov/protein/NP_851337.1)

<sup>20</sup> Technical dossier/p. 39; Brenda Database.

<sup>21</sup> Technical dossier/Additional information, 1 September 2021/ [https://www.ncbi.nlm.nih.gov/protein/XP\\_005699798.1](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/XP_004019629.2); [https://www.ncbi.nlm.nih.gov/protein/NP\\_001001600.2](https://www.ncbi.nlm.nih.gov/protein/NP_001001600.2)

<sup>22</sup> Technical dossier/Additional information, 1 September 2021/ [https://www.ncbi.nlm.nih.gov/protein/XP\\_017900376.1](https://www.ncbi.nlm.nih.gov/protein/XP_017900376.1); [https://www.ncbi.nlm.nih.gov/protein/XP\\_027816132.2](https://www.ncbi.nlm.nih.gov/protein/XP_027816132.2); [https://www.ncbi.nlm.nih.gov/protein/NP\\_776528.1](https://www.ncbi.nlm.nih.gov/protein/NP_776528.1)

<sup>23</sup> Technical dossier/p. 40; UniProtKB database.

<sup>24</sup> Technical dossier/p. 10, 34, 41.

<sup>25</sup> Technical dossier/p. 38.

<sup>26</sup> Technical dossier/p. 40; Technical dossier/Annex 7.

<sup>27</sup> Technical dossier/Annex 8.

<sup>28</sup> Technical dossier/p. 41; Technical dossier/Annex 9.



The rennet paste milk clotting activity has a pH optimum around 6.5 and a temperature optimum around 45°C. This activity decreased above 50°C showing no residual activity above 55°C.<sup>29</sup>

The rennet paste triacylglycerol lipase activity has a pH optimum around pH 6.0 (30°C) and a temperature optimum around 40°C (pH 5.5). This activity decreased above 50°C, showing no residual activity above 65°C.<sup>30</sup>

### 3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (Table 1). The mean total organic solids (TOS) is 16.6% and the mean enzyme clotting activity/mg TOS ratio is 0.8 IMCU/mg TOS.<sup>31</sup>

**Table 1:** Composition of the food enzyme rennet paste from the abomasum of suckling goats, lambs and calves (different producers and processes)

Parameters	Unit	Batches		
		1	2	3
<b>Milk clotting activity</b>	IMCU/g batch <sup>(a)</sup>	115	131	128
<b>Triacylglycerol lipase activity</b>	ILU/g <sup>(b)</sup>	6	5	5.6
<b>Protein</b>	%	6.3	9.2	5.0
<b>Ash</b>	%	21.7	32.7	20.8
<b>Water</b>	%	58.4	47.9	68.8
<b>Total organic solids (TOS)<sup>(c)</sup></b>	%	19.9	19.4	10.4
<b>Triacylglycerol lipase activity/mg TOS</b>	ILU/mg TOS	0.03	0.03	0.05
<b>Milk clotting activity/mg TOS</b>	IMCU/mg TOS	0.6	0.7	1.2

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

(b): ILU: International Lipase Unit (see Section 3.3.1).

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

### 3.3.3. Purity<sup>32</sup>

The lead content in the three commercial batches 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).<sup>33,34</sup> In addition, the levels of mercury, cadmium and arsenic were below the limits of quantification of the employed methodologies.<sup>35,36</sup>

The food enzyme complies with the microbiological criteria (for total coliforms, *Escherichia coli* and *Salmonella*) as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).<sup>37</sup> *Listeria* spp. was analysed in 25 g of three representative commercial batches (one each of suckling goat, lamb and calf rennet paste) (ISO method 11290-1:2017). The results raised no concern.<sup>12</sup>

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

## 3.4. Toxicological data<sup>38</sup>

According to the Commission Implementing Regulation (EU) No 562/2012<sup>39</sup>, 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.

<sup>29</sup> Technical dossier/p. 41–42.

<sup>30</sup> Technical dossier/p. 43.

<sup>31</sup> Technical dossier/p. 37; Technical dossier/Annex 1, Annex 2, Annex 3, Annex 4, Annex 5, Annex 6.

<sup>32</sup> Technical dossier/p. 10; 38–39, 80; Technical dossier/Annex 10, Annex 11, Annex 12, Annex 17, Annex 18; Technical dossier/Additional information, 1 September 2021.

<sup>33</sup> Technical dossier/p. 10; 38–39; Technical dossier/Annex 1, Annex 2, Annex 3, Annex 18.

<sup>34</sup> LOQ: Pb = 0.01 mg/kg.

<sup>35</sup> Technical dossier/Annex 1, Annex 2, Annex 3.

<sup>36</sup> LOQs: As, Hg = 0.05 mg/kg (each); LOQ: Cd = 0.01 mg/kg.

<sup>37</sup> Technical dossier/p. 10; 38–39; Technical dossier/Annex 10, Annex 11, Annex 12, Annex 17.

<sup>38</sup> Technical dossier/p. 14–15, 20, 45, 67.

<sup>39</sup> 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.



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 considers that these requirements are fulfilled, because:

- (i) rennet obtained from the abomasum of suckling goats, lambs and calves has been safely used in the production of cheese and related products for many centuries;
- (ii) the abomasum from suckling goats, lambs and calves is consumed throughout the EU and elsewhere in the world as meat product<sup>40</sup>;
- (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 considers 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 is waived.

### 3.4.1. Allergenicity<sup>41</sup>

The potential allergenicity of the food enzyme containing chymosin, pepsin and triacylglycerol lipase derived from the abomasum of suckling goats, lambs and calves was not assessed by comparing its amino acid sequence with those of known allergens and not considered relevant in this case.

Occupational respiratory allergies and skin sensitisation to dust of chymosin and pepsin have been described in workers upon industrial exposure and in medical laboratory technicians (Cartier et al., 1984; Jensen et al., 2006; van Kampen et al., 2013; Gómez Torrijos et al., 2018; Khan and Selamoglu, 2020). Occupational allergies to triacylglycerol lipase have also been described (Elms et al., 2003; Lindstedt et al., 2005; Shin et al., 2008; Loureiro et al., 2009; Martel et al., 2010; Budnik et al., 2017). 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 (Cullinan et al., 1997; Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). There are no reports in the literature on adverse reactions upon ingestion of these enzymes in individuals sensitised through the respiratory route.

No information is available on oral sensitisation or elicitation reactions to chymosin, pepsin A and triacylglycerol lipase obtained from the abomasum of suckling goats, lambs and calves under evaluation.<sup>12</sup>

The Panel notes that milk proteins are present in the rennet paste. However, as the paste is used in cheese processing this will not pose an additional risk to cheese consumption.

The Panel considers that the likelihood of food allergic reactions to this food enzyme obtained from the abomasum of suckling goats, lambs and calves is low and, therefore, does not give rise to safety concerns under the intended conditions of use.

## 3.5. Dietary exposure

### 3.5.1. Intended use of the food enzyme

The food enzyme (rennet paste) is intended to be used in milk processing for cheese production at the recommended use level from around 1 to 16 mg TOS/kg milk.<sup>42</sup>

Animal rennet is added to milk to separate milk into solid curd and liquid whey (coagulation). Both chymosin and pepsin contribute to the milk-clotting activity.<sup>43</sup> The majority of the food enzyme TOS partitions into the whey and is mostly removed during the draining of the whey.<sup>44</sup> Only a small portion of the food enzyme TOS remains in the curd (~ 6–12%). The remaining rennet contributes to the ripening of cheese due to its general proteolytic activity.<sup>44</sup>

Based on data provided on thermostability (see Section 3.3.1), it is expected that the remaining food enzyme is inactivated during cheese making.

<sup>40</sup> Technical dossier/p. 45.

<sup>41</sup> Technical dossier/p. 15, 67–69; Technical dossier/Additional information, 1 September 2021.

<sup>42</sup> Technical dossier/p. 13, 30, 60.

<sup>43</sup> Technical dossier/p. 9–10, 13, 20.

<sup>44</sup> Technical dossier/p. 57, 84–85.

### 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, abomasum from ruminants is consumed in some European countries, which constitutes a minor fraction of the overall exposure to the food enzyme in the EU.

In the view of the Panel dietary exposure estimation is not required.

### 3.6. Margin of exposure

Since no toxicological assessment and no dietary exposure estimation were considered necessary by the Panel, 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 concludes that the food enzyme rennet paste containing chymosin, pepsin A and triacylglycerol lipase obtained from the abomasum of suckling goats, lambs and calves does not give rise to safety concerns under the intended conditions of use.

## 5. Documentation as provided to EFSA

- 1) Technical dossier 'Application for authorisation of rennet paste from abomasum (fourth stomach) of *Capra aegagrus hircus* (goat), *Ovis aries* (sheep) and *Bos primigenius* (cattle) in accordance with Regulation (EC) No 1331/2008'. 23 February 2015. Submitted by Caglificio Clerici S.p.A.
- 2) Additional information. 1 September 2021. Submitted by Caglificio Clerici S.p.A.
- 3) Summary report on technical data and dietary exposure. 22 February 2016. Delivered by contractor Hylobates Consulting and BiCT (Rome, Italy).

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## Abbreviations

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
HPLC	high-performance liquid chromatography
IDF	International Dairy Federation
ILU	International Lipase Unit
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
LOQ	limit of quantification
TOS	total organic solids
WHO	World Health Organization