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Safety evaluation of the food enzyme containing chymosin and pepsin from the abomasum of suckling lambs and goats

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Abstract

The food enzyme containing chymosin (EC 3.4.23.4) and pepsin (EC 3.4.23.1) is derived from the abomasum of suckling lambs and goats by Caporal Enzymes, S.L. 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 considered that toxicological data were not required and no exposure assessment was necessary. On the basis of literature data, the Panel considered 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 for this to occur was considered to be low. Based on the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

Keywords: food enzyme, chymosin, EC 3.4.23.4, pepsin, EC 3.4.23.1, abomasum, suckling lamb, suckling goat

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1. Introduction

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

> 'Food enzyme' means a product obtained from plants, animals or micro-organisms or products thereof including a product obtained by a fermentation process using micro-organisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.
>
> 'Food enzyme preparation' means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

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

An application has been introduced by the applicant 'Caporal Enzymes, S.L.' for the authorisation of the food enzyme preparation chymosin and pepsin from stomachs of lambs and kids (young goats).

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 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 assessment on the following food enzyme preparation chymosin and pepsin from stomachs of lambs and kids (young goats) 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, p. 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 containing chymosin and pepsin from the abomasum of suckling lambs and goats.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme containing chymosin and pepsin from the abomasum of suckling lambs and goats.

Additional information was requested from the applicant during the assessment process on 3 March 2020 and was consequently 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, 2009) and following the relevant existing guidances 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[4]

The food enzyme under application contains two declared activities[5]:

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 κ-casein. This results in extensive 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, breaks down peptide bonds in protein and peptide molecules with the formation of shorter peptides, and free amino acids. It preferably cleaves peptide bonds between hydrophobic and aromatic amino acids.

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

[4] Technical dossier/3.2.1. Technical data/p. 1–3; Technical dossier/Additional data, 17 February 2021/1.1. Technical data/p. 1–17.
[5] Technical dossier/3.2.1. Technical data/p. 1; Technical dossier/Additional data, 17 February 2021/1.1. Technical data/p. 1–17.

3.1. Source of the food enzyme[6]

The food enzyme is extracted from the abomasum of suckling lambs (*Ovis aries*) and suckling goats (*Capra aegagrus hircus*). The raw materials are stomachs/abomasums[7] coming from slaughterhouses registered or approved under the Food Hygiene Regulation (EC) No 852/2004[8] and Regulation (EC) No 853/2004[9]. Examples of certificates from slaughterhouses were provided by the applicant confirming that animal tissues used for the preparation of food enzymes comply with meat inspection requirements and are handled in accordance with good hygienic practice.[10]

In EU, according to Regulation (EC) No 1774/2002[11], the stomach (abomasum) of lambs and goats is considered fit for human consumption, as it is not on the list of animal by-products that are unfit for human consumption. It is an edible offal as defined in Regulation (EC) No 853/2004[9].

3.2. Production of the food enzyme[12]

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[8], with food safety procedures based on hazard analysis and critical control points, and in accordance with current Good Manufacturing Practice.

The applicant describes the production of three products, a lamb liquid rennet (standard and soft), a goat liquid rennet, and lamb rennet paste.[13]

For the production of liquid rennet, degreased abomasum of suckling lambs and goats [redacted], are used as raw material. The abomasums are [redacted].[14]

For the production of rennet paste the degreased abomasums [redacted].[15]

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.[16]

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme[17]

3.3.1.1. Properties of chymosin and pepsin from the abomasum of suckling lambs[18]

The chymosin from the abomasum of suckling lambs is a single polypeptide chain of [redacted][19] amino acids. The molecular mass of the mature protein was calculated to be [redacted] kDa.[20] The pepsin A from the abomasum of suckling lambs is a single polypeptide chain of [redacted][19] amino acids. The molecular mass of the mature protein was calculated to be [redacted] kDa.[20]

The food enzyme lamb rennet was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis (six batches of liquid lamb rennet and six batches of the lamb

[6] Technical dossier/3.2.1. Technical data/p. 10–12; Technical dossier/3.1 Administrative data/Document No. 8 and 9.
[7] Technical dossier/Risk management data; Technical dossier/3.2.1. Technical data/p. 10; Technical dossier/3.1 Administrative data/Document No. 8 and 9.
[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] Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs. OJ L139/55, 30.4.2004.
[10] Technical dossier/3.1 Administrative data/Document No. 8 and 9.
[11] 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 L273, 10.10.2002.
[12] Technical dossier/3.2.1. Technical data/p. 12–22; Technical dossier/Additional data, 17 February 2021/2. Manufacturing process.
[13] Technical dossier/3.3. Risk management data/p. 1.
[14] Technical dossier/3.2.1. Technical data/p. 12–23.
[15] Technical dossier/3.2.1. Technical data/p. 16–21.
[16] Technical dossier/3.2.1. Technical data/p. 13, 16.
[17] Technical dossier/3.2.1. Technical data/p. 3–4, 8–10; Technical dossier/Additional data, 17 February 2021/1.1. Technical data.
[18] Technical dossier/3.2.1. Technical data/p. 9–10; Technical dossier/Additional data, 17 February 2021/1.1. Technical data.
[19] Technical dossier/Additional data, 17 February 2021/1.1. Technical data/p. 3–11.
[20] Technical dossier/Additional data, 17 February 2021/1.1. Technical data/p. 3–11/UniProt.

paste rennet).[21] A consistent main band was observed for all samples with a molecular mass of the same order of magnitude of the value reported for chymosin. This band was further analysed by mass spectrometry. The main protein found was chymosin precursor with molecular mass [redacted] kDa.[22]

The determination of chymosin and pepsin activities is based on the official method ISO 23058 IDF 199:2006.[23] 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 the rennet sample is compared to that of a bovine rennet reference standard with a defined enzyme composition of 75:25 and with known milk-clotting activity. The total milk-clotting activity of both enzymes is expressed in IMCUS (International Milk-Clotting Units).

Literature data on a temperature optimum, a pH optimum and thermostability of chymosin from the abomasum of lambs are not available.[24] Literature data for recombinant lamb chymosin showed that an optimal clotting activity was obtained at 40°C. The temperature instability of recombinant lamb chymosin occurs at temperatures above 45°C (Rogelj et al., 2001).

The general proteolytic pH optimum of chymosin is about 3.8, but it has high specific milk clotting activity at the pH of milk, that is 6.7 (Andrén, 2011).

Literature data on temperature optimum and thermostability of pepsin from the abomasum of suckling lambs are not available.[24] Pepsin has its general proteolytic pH optimum at about 2 (Andrén, 2011).

3.3.1.2. Properties of chymosin and pepsin from the abomasum of suckling goats[25]

The chymosin from the abomasum of suckling goats is a single polypeptide chain of [redacted][26] amino acids. The molecular mass is [redacted][26] kDa (Kumar et al., 2006; Moschopoulou et al., 2006). The applicant did not provide reference to the amino acid sequence of pepsin from *Capra hircus.* The molecular mass of the purified pepsin from the abomasum of suckling goats as determined by gel filtration is [redacted][27] kDa (Moschopoulou et al., 2006). The rennet was analysed by SDS–PAGE analysis (three batches). A consistent main band was observed for all samples with a molecular mass of the same order of magnitude of the value reported for chymosin. This band was further analysed by mass spectrometry. The main protein found in was prochymosin with molecular mass [redacted] kDa.[28]

The determination of chymosin and pepsin activities is based on the official method ISO 23058 IDF 199:2006.[23] 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 the rennet sample is compared to that of a bovine rennet reference standard with a defined enzyme composition of 75:25 and with known milk-clotting activity. The total milk-clotting activity of both enzymes is expressed in IMCUS.

The chymosin from the abomasum of suckling goats has a temperature optimum around 35°C (20–38°C) and a pH optimum around pH 5.5 (pH 5.0–6.5).[29] The enzyme shows more than 90% activity over a temperature range of 30–45°C and about 75% activity at 20°C and 50°C.[29] The enzyme retains about 65%, 100%, 85% and 69% of milk clotting activity at pH 5.0, 5.5, 6.0 and 6.5, respectively.[29]

3.3.2. Chemical parameters[30]

3.3.2.1. Chemical parameters for lamb and goat liquid rennets[23]

Data on the chemical parameters were provided for five batches of lamb liquid rennet and for two batches of goat liquid rennet (Table 1).

[21] Technical dossier/3.1 Administrative data/Document 2; Technical dossier/Additional data, 17 February 2021/1.1. Technical data and 1.2. Technical data.
[22] Technical dossier/Additional data, 17 February 2021/1.1. Technical data/p. 20.
[23] Technical dossier/Additional data, 17 February 2021/1.3. Technical data.
[24] Technical dossier/Additional data, 17 February 2021/1.1. Technical data/p. 10.
[25] Technical dossier/3.2.1. Technical data/p. 3–4, 8–10; Technical dossier/Additional data, 17 February 2021/1.1. Technical data/p. 12–13.
[26] Technical dossier/Additional data, 17 February 2021/1.1. Technical data/p. 12–13.
[27] Technical dossier/Additional data, 17 February 2021/1.1. Technical data/p. 12.
[28] Technical dossier/Additional data, 17 February 2021/1.1. Technical data/p. 19.
[29] Technical dossier/Additional data, 17 February 2021/1.1. Technical data/p. 16.
[30] Technical dossier/3.2.1. Technical data/p. 3–4; Technical dossier/Additional data, 17 February 2021/1.3. Technical data.

Table 1: Chemical composition of the liquid rennet from the abomasum of suckling lambs and goats

Parameters	Unit	Lamb liquid rennet (five batches)		Goat liquid rennet (two batches)	
		Mean[(a)]	Minimum–Maximum	1	2
Rennet activity	IMCUS/mL batch[(b)]	123.3	111.5–133.0	96.3	106.9
Protein	%	2.6	2.3–3.1	2.0	2.3
Ash	%	12.6	12.1–13.2	12.2	12.4
Water	%	83.1	81.8–84.0	83.8	83.6
Total organic solids (TOS)[(c)]	%	4.4	3.6–5.0	4.0	4.0
Rennet activity/mL TOS	IMCUS/mL TOS	2.9	2.6–3.1	2.4	2.7

(a): Mean values of five commercial batches of lamb liquid rennet.
(b): IMCUS: International Milk-Clotting Units (see Section 3.3.1).
(c): TOS calculated as 100% – % water – % ash.

3.3.2.2. Chemical parameters for lamb rennet paste[31]

The same chemical parameters were analysed in three batches of lamb rennet paste (Table 2). The average total organic solids (TOS) was 26.8%. The average rennet activity was 142 IMCUS/mL, as determined in another three batches (not shown in Table 2).[32]

Table 2: Chemical composition of the lamb rennet paste

Parameter	Unit	Batches		
		1	2	3
Rennet activity	IMCUS/mL batch[(a)]	NA	NA	NA
Protein	%	8.9	7.7	5.2
Ash	%	43.1	48.9	49.8
Water	%	29.2	32.1	16.4
Total Organic Solids (TOS)[(b)]	%	27.7	19.0	33.8
Rennet activity/mL TOS	IMCUS/mL TOS	NA	NA	NA

NA: not available.
(a): IMCUS: International Milk-Clotting Units (see Section 3.3.1).
(b): TOS calculated as 100% – % water – % ash.

3.3.3. Purity[33]

The lead content in the seven commercial batches (three batches of lamb and one batch of goat liquid rennet, three batches of lamb rennet paste)[34] was below 5 mg/kg which complies with the specification for lead (≤ 5 mg/kg) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006). In addition, the levels of arsenic, mercury and cadmium were below the limits of detection (LODs) of the employed methodologies.[35]

The food enzyme preparation complies with the microbiological criteria (for total coliforms, *Escherichia coli* and *Salmonella*) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006). In addition, the results of the analysis of aerobic bacteria, Enterobacteriaceae, *Listeria*, filamentous fungi and yeasts, staphylococci and *Clostridium* spp. were reported and did not raise any safety concern.[36]

[31] Technical dossier/Additional data, 17 February 2021/1.3. Technical data/p. 115–117.
[32] Technical dossier/Additional data, 17 February 2021/1.3. Technical data/p. 35–69.
[33] Technical dossier/3.2.1. Technical data/p. 5–6; Technical dossier/Additional data, 17 February 2021/1.3. Technical data.
[34] Technical dossier/3.2.1. Technical data/p. 5–6; Technical dossier/3.1 Administrative data/Document No. 4; Technical dossier/Additional data, 17 February 2021/1.3. Technical data.
[35] Technical dossier/3.2.1. Technical data/p. 5–6/LODs: Pb = 0.01432 μg/kg; As = 0.1226 μg/kg; Cd = 0.009331 μg/kg; Hg = 0.07686 μg/kg.
[36] Technical dossier/3.2.1. Technical data/p. 6–7; Technical dossier/3.1 Administrative data/Document No. 5 and 6; Technical dossier/Additional data, 17 February 2021/1.3. Technical data.

The presence of aflatoxin B1, B2, G1, G2 and M1[37] was examined in three food enzyme preparation batches and were below the LODs of the applied analytical methods.[38]

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

3.4. Toxicological data[39]

According to the Commission Implementing Regulation (EU) No 562/2012[40], 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 suckling lamb and goat abomasum has been safely used in the production of cheese and related products for many centuries;
ii) the abomasum from suckling lambs and goats is consumed throughout the EU and elsewhere in the world as a meat product[41];
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 for the food enzyme preparations 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 is waived.

3.4.1. Allergenicity[42]

The potential allergenicity of the food enzyme containing chymosin and pepsin derived from the abomasum of suckling lambs and goats was not assessed by comparing its amino acid sequence with those of known allergens. 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). 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.

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

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

[37] Technical dossier/3.2.1. Technical data/p. 6; Technical dossier/3.1 Administrative data/Document No. 3.
[38] Technical dossier/3.2.1. Technical data/p. 6/LODs of mycotoxins: aflatoxin M1: 10 ng/L; aflatoxin G2: 50 ng/kg; aflatoxin G1: 75 ng/kg; aflatoxin B2: 50 ng/kg; aflatoxin B1: 50 ng/kg.
[39] Technical dossier/3.2.1. Technical data/p. 30.
[40] 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.
[41] Technical dossier/Additional data, 17 February 2021/3.1. Allergenicity.
[42] Technical dossier/3.2.1. Technical data/p. 30–31; Technical dossier/Additional data, 17 February 2021/3.1. Allergenicity.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme[43]

The food enzyme (liquid rennet) is intended to be used in milk processing for cheese production at the recommended use level of about 30 mg TOS/kg milk.[44] No use level was provided for the paste rennet.

Animal rennet is added to milk to separate milk into solid curd and liquid whey (coagulation).[45] The majority of the food enzyme–TOS partitions into the whey. The rennet is mostly removed during the draining of the whey and only a small portion remains in the curd (approximately 6–12%).[46] The remaining rennet contributes to the ripening of cheese due to its general proteolytic activity.

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 EU.

In the view of the Panel and taking the weight of evidence approach, dietary exposure estimation is not required.

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 containing chymosin and pepsin from the abomasum of suckling lambs and goats does not give rise to safety concerns under the intended conditions of use.

5. Documentation as provided to EFSA

1) Technical dossier `Chymosin and pepsin from stomachs of lambs and kids (young goats)´. 9 March 2015. Submitted by Caporal Enzymes, S.L.
2) Additional information. 17 February 2021. Submitted by Caporal Enzymes, S.L.

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[43] Technical dossier/3.2.1. Technical data/p. 24–27.
[44] Technical dossier/Risk assessment data/p. 26.
[45] Technical dossier/Risk management data/p. 3.
[46] Technical dossier/3.2.1. Technical data/p. 24.

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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
IMCUS	International Milk-Clotting Units
ISO	International Organization for Standardization
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	total organic solids
WHO	World Health Organization