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Safety evaluation of a food enzyme containing chymosin, pepsin and gastricsin from the abomasum of suckling goats

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Abstract

The food enzyme containing chymosin (EC 3.4.23.4), pepsin (EC 3.4.23.1) and gastricsin (EC 3.4.23.3) is prepared from the abomasum of suckling goats by Consejo Regulador de la Denominación de Origen Queso Palmero and Consejo Regulador de la Denominación de Origen Queso Majorero. 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. Similarity of the amino acid sequences of the three proteins (chymosin, pepsin and gastricsin) to those of known allergens was searched and one match was found. The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but 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, EC 3.4.23.1, gastricsin, EC 3.4.23.3, abomasum, suckling goats

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† Deceased.

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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 'Instituto Canario de Calidad Agroalimentaria, Las Palmas de Gran Canaria' for the authorisation of the food enzyme preparation chymosin, pepsin and gastricsin from stomachs (abomasum) of kids (young goats).

Following the requirements of Article 12.1 of Commission Regulation (EU) 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 (EC) requests the European Food Safety Authority (EFSA) to carry out the safety assessment on the following food enzyme preparation chymosin, pepsin and gastricsin from stomachs (abomasum) of 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, 11.03.2011, 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 a food enzyme containing chymosin, pepsin and gastricsin from the abomasum of suckling 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, pepsin and gastricsin from the abomasum of suckling goats.

Additional information was requested from the applicant during the assessment process on 14 April 2020 and 15 February 2021 and received on 21 December 2020 and 1 July 2021 (see 'Documentation as 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 with 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 three declared activities: chymosin, pepsin and gastricsin[5]:

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

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

IUBMB nomenclature	Gastricsin
Synonyms	Pepsin C; parapepsin II
IUBMB No.	3.4.23.3
CAS No.	9012-71-9
EINECS No.	232-645-0

[4] Dossier Kid rennet complete/p. 6–7, 10; 14–15; 22–23, 55; 3. Technical dossier/p. 9–10; 17–18; 42; 2.1. Summary of the dossier/p. 1–3.

[5] Dossier Kid rennet complete/p. 6–7, 10; 14–15; 22–23, 55; 2.1. Summary of the dossier/p. 1.

Gastricsin is also an aspartic endopeptidase, with more restricted specificity than pepsin A, but shows preferential cleavage adjacent to tyrosine residues.

The food enzyme is intended to be used in milk processing for cheese production.[6]

3.1. Source of the food enzyme[7]

The food enzyme is obtained from the abomasum of suckling goats from the species *Capra hircus*[8] from certified European suppliers, 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[9] and Regulation (EC) No 853/2004.[10,11]

In EU, according to Regulation (EC) 1774/2002[12], stomach (abomasum) of goats is considered fit for human consumption. It is an edible offal as defined in Regulation (EC) No 853/2004[10], but it has not been reported to be commonly consumed in the European Union. Stomach of goats has been considered an edible part of a goat in the US (Marti et al., 2011; Nollet and Toldra, 2011) and Australia (FSANZ, 2016).

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

3.2. Production of the food enzyme[13]

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[9], with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[14]

The food enzyme is extracted from the abomasum of suckling goats. After the animal is slaughtered, the abomasum is collected with its content (in some cases, the abomasa are intentionally filled with milk), salted (storage in brine or salt layers) and air-dried. The dry abomasa with content are then cut, ground, mixed with salt and homogenised. The applicant provided information on the identity of the substances used in the extraction and in the subsequent downstream processing.[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.

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme[16]

The chymosin is a single polypeptide chain of 366 amino acids.[17] The molecular mass was calculated to be 36.0 kDa.[18] The pepsin A is a single polypeptide chain of 386 amino acids.[19] The molecular mass was calculated to be 40.0 kDa.[20] The gastricsin is a single polypeptide chain of 391 amino acids.[21] The molecular mass was calculated to be 39.0 kDa.[22]

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[6] Technical dossier/Additional information, 21 December 2020.
[7] Dossier Kid Rennet Complete/p. 6–7, 26–27; 32–33; 3. Technical dossier/p. 13; 19–20.
[8] 3. Technical dossier/p. 13.
[9] 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.
[10] 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.
[11] Dossier Kid Rennet Complete/p. 36; 3. Technical dossier/p. 23.
[12] 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.
[13] Dossier Kid Rennet Complete/p. 33–40; 3. Technical dossier/p. 24–27; Technical dossier/Additional information, 21 December 2020.
[14] Technical dossier/Additional information, 21 December 2020/p. 3–5 and Annex 1.
[15] Dossier Kid Rennet Complete/p. 33–40; 3. Technical dossier/p. 24–27.
[16] Dossier Kid Rennet Complete/p. 29–32; 3. Technical dossier/p. 16–19; Technical dossier/Additional information, 1 July 2021/ Annex 1.1.
[17] Dossier Kid Rennet Complete/p. 24–25; 3. Technical dossier/p. 11–12.
[18] Dossier Kid Rennet Complete/p. 24; 3. Technical dossier/p. 11; Technical dossier/Additional information, 21 December 2020.
[19] Dossier Kid Rennet Complete/p. 25; 3. Technical dossier/p. 12.
[20] Dossier Kid Rennet Complete/p. 24; 3. Technical dossier/p. 11–12; Technical dossier/Additional information, 21 December 2020.
[21] Dossier Kid Rennet Complete/p. 25–26; 3. Technical dossier/p. 13.
[22] Dossier Kid Rennet Complete/p. 26, 3. Technical dossier/p. 11, 13; Technical dossier/Additional information, 21 December 2020.

The determination of chymosin and pepsin activities is based on the official method ISO 11815|IDF 157:2007, 2007 for bovine rennet and ISO 23058|IDF 199:2006, 2006 for sheep and goat rennet.[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 known milk-clotting activity. The total milk-clotting activity of both enzymes is expressed in IMCU (International Milk-Clotting Units).

The relative contents of chymosin and pepsin present in the rennet were determined by chromatographic analysis, based on the recognised official method ISO 15163|IDF 110:2012, 2012 for milk and milk products, indicated for calf rennet and adult bovine rennet. The analysis of gastricsin was not considered of relevance as it is found only in low concentrations in the stomach of young goats.

Literature data showed that the kid chymosin has a temperature optimum around 48–52°C (pH 6.6) and a pH optimum around pH 6 (at 35°C).[6] The kid pepsin has a temperature optimum around 44–46°C (pH 6.6) and a pH optimum around pH 6 (at 35°C).[6] The kid gastricsin has a pH optimum around pH 3 (at 37°C).[6] Literature data on a temperature optimum of gastricsin from the abomasum of suckling goats are not available.

Literature data on thermostability showed that chymosin becomes inactive at temperature above 60°C (at pH 6.2), 56°C (at pH 6.4) and 52°C (at pH 6.6) and pepsin A becomes inactive at temperature above 56°C (at pH 6.2), 52°C (at pH 6.4) and 46°C (at pH 6.6) (Moschopoulou et al., 2006).[6]

3.3.2. Chemical parameters[24]

Analytical data on the enzyme activity were provided by the applicant for 5 batches (batches 1–5) of rennet from the abomasum of suckling goats (Table 1). Data on other chemical parameters of the food enzyme were provided for 25 batches (batches 6–30) of rennet used for commercialisation (Table 1). The average total organic solids (TOS) of the 25 food enzyme batches was 24 ± 8%.

Table 1: Compositional data of the rennet from the abomasum of suckling goats

Parameter	Unit	Batches 1–5 (mean ± SD)	Batches 6–30 (mean ± SD)
Enzyme activity (official method ISO 1815|IDF 157:2007, 2007)	IMCU/g batch[(a)]	91 ± 39	NA[(c)]
Enzyme activity (official method ISO 23058|IDF 199:2006, 2006)	IMCU/g batch[(a)]	88 ± 38	NA
Protein	%	–	NA
Ash	%	–	21 ± 5
Water	%	–	56 ± 6
Total organic solids (TOS)[(b)]	%	–	24 ± 8
Activity/mg TOS	IMCU/mg TOS	–	NA

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

The chromatographic analysis of the two main enzymes present in the rennet (chymosin and pepsin) for five analysed samples showed an average value (± SD) of 91 ± 5% for chymosin and 9 ± 5% for pepsin, respectively.[25] As the third declared activity, gastricsin, is present only in low concentrations in the abomasum of suckling goats (Addis et al., 2008; Moschopoulou, 2011), it was not included in the analysis made by the applicant.[23]

[23] Technical dossier/Additional information, 1 July 2021/Annex 1.1.
[24] Technical dossier/Additional information, 21 December 2020; Technical dossier/Additional information, 1 July 2021/Annex 1.1, Annex 1.2.
[25] Technical dossier/Additional information, 1 July 2021/p. 1–2.

3.3.3. Purity[26]

The lead content[27] in the 25 commercial batches was below 0.03 mg/kg 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). In addition, the levels of arsenic, cadmium and mercury[27] were below the limits of detection of the employed methodologies.[28]

The microbiological analyses of 25 commercial batches were reported. 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). Additional microbiological parameters (sulfite-reducing clostridia, enterococci, *Pseudomonas* spp., *Listeria* spp., fungi, yeasts) were also reported and raised no concern.[29]

3.4. Toxicological data[30]

According to the Commission Implementing Regulation (EU) No 562/2012[31], 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 considers that these requirements are fulfilled, because:

i) Rennet obtained from the abomasum of suckling goats has been safely used in the production of cheese and related products for many centuries;
ii) The abomasum from suckling goats is consumed throughout the EU and elsewhere in the world as a meat product;
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 is waived.

3.4.1. Allergenicity[32]

The potential allergenicity of the food enzyme containing chymosin, pepsin A and gastricsin derived from the abomasum of suckling goats 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, one match was found. The matching allergen was pig pepsin[33] known as an occupational respiratory allergen.

Occupational respiratory allergies and skin sensitisation to dust of chymosin, pepsin A and gastricsin 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

[26] Technical dossier/Additional information, 21 December 2020/Annex III; Table 1; Table 3; Technical dossier/Additional information, 1 July 2021/Annex 1.2.
[27] Technical dossier/Additional information, 21 December 2020/Annex III and Table 1.
[28] Technical dossier/Additional information, 21 December 2020/Annex III; LODs: Pb = 1 ng/L; As = 1 ng/L; Cd = 1 ng/L; Hg = 1 ng/L.
[29] Technical dossier/Additional information, 21 December 2020/Table 3; Technical dossier/Additional information, 1 July 2021/Annex 1.2.
[30] Dossier Kid Rennet Complete/p. 44–45; 3. Technical dossier/p. 31–32.
[31] 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.
[32] Dossier Kid Rennet Complete/p. 45–50; 3. Technical dossier/p. 32–37; Technical dossier/Additional information, 21 December 2020.
[33] Dossier Kid Rennet Complete/p. 49; 3. Technical dossier/p. 36.

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 goat rennet paste. However, as the paste is used in cheese processing this will not pose an additional risk to cheese consumption.

No information is available on oral sensitisation or elicitation reactions to this chymosin, pepsin and gastricsin obtained from the abomasum of suckling goats under evaluation.

Goat is not a source included in the list of substances or products causing allergies or intolerances (EU Regulation 1169/2011).[34]

Consequently, the Panel considers that the likelihood of food allergic reactions to this food enzyme obtained from the abomasum of suckling goats 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 and by-products at a use level of 10–20 g rennet/100 L of milk, corresponding to 23.7–47.5 mg TOS/kg milk.[6]

The food enzyme preparation is added to milk to separate milk into solid curd and liquid whey (coagulation).[6] 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 (approximately 6–12%).[35] The remaining rennet contributes to the ripening of cheese due to its general proteolytic activity.

Based on data provided on thermostability (see Section 3.3.1), it is expected that the remaining chymosin and pepsin are inactivated during cheese making.

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, 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 concluded that the food enzyme containing chymosin, pepsin and gastricsin from abomasum of suckling goats does not give rise to safety concerns under the intended conditions of use.

5. Documentation as provided to EFSA

1) Technical dossier `Food enzyme preparation chymosin, pepsin and gastricsin from stomachs (abomasum) of kids (young goats)‘. 9 March 2015. Submitted by Consejo Regulador de la

[34] 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. L 304, p. 46, 22.11.2011.

[35] 3. Technical dossier/p. 27.

Denominación de Origen Queso Palmero and Consejo Regulador de la Denominación de Origen Queso Majorero (Instituto Canario de Calidad Agroalimentaria, Las Palmas de Gran Canaria).

2) Additional information. 21 December 2020. Submitted by Consejo Regulador de la Denominación de Origen Queso Palmero and Consejo Regulador de la Denominación de Origen Queso Majorero (Instituto Canario de Calidad Agroalimentaria, Las Palmas de Gran Canaria).
3) Additional information. 1 July 2021. Submitted by Consejo Regulador de la Denominación de Origen Queso Palmero and Consejo Regulador de la Denominación de Origen Queso Majorero (Instituto Canario de Calidad Agroalimentaria, Las Palmas de Gran Canaria).

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Abbreviations

CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
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
FSANZ	Food Standards Australia New Zealand
GM	genetically modified
GMO	genetically modified organism
IDF	International Diary Federation
IMCU	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
NA	not analysed
SD	standard deviation
TOS	total organic solids
WHO	World Health Organization