

ADOPTED: 15 November 2022

doi: 10.2903/j.efsa.2022.7681

Safety evaluation of the food enzyme phytepsin from *Cynara cardunculus* L.

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP),
Claude Lambré, José Manuel Barat Baviera, Claudia Bolognesi, Pier Sandro Cocconcelli,
Riccardo Crebelli, David Michael Gott, Konrad Grob, Evgenia Lampi, Marcel Mengelers,
Alicja Mortensen, Gilles Rivière, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren,
Laurence Vernis, Holger Zorn, Yrjö Roos, Magdalena Andryszkiewicz, Yi Liu and
Andrew Chesson

Abstract

The food enzyme phytepsin (EC 3.4.23.40) is extracted from the pistils of the cardoon *Cynara cardunculus* L. by ABIASA. It is intended to be used in milk processing for cheese production. As no concerns arose from the source of the food enzyme, 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. The Panel considered that allergic reactions to this phytepsin cannot be excluded in individuals allergic to this plant. However, the likelihood of allergic reactions to the phytepsin from *C. cardunculus* L. is expected not to exceed the likelihood of allergic reactions to cardoon. As the prevalence of allergic reactions to cardoon is low, also the likelihood of such reaction to occur to the food enzyme 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.

© 2022 Wiley-VCH Verlag GmbH & Co. KGaA on behalf of the European Food Safety Authority.

Keywords: food enzyme, plant coagulant, phytepsin, EC 3.3.23.40, cardoon, flower, *Cynara cardunculus* L.

Requestor: European Commission

Question number: EFSA-Q-2016-00271

Correspondence: fip@efsa.europa.eu

Panel members: José Manuel Barat Baviera, Claudia Bolognesi, Andrew Chesson, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Claude Lambré, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis and Holger Zorn.

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.

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

Acknowledgements: The CEP Panel wishes to thank the following for the support provided to this scientific output: Erik Boinowitz.

Suggested citation: EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré C, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mengelers M, Mortensen A, Rivière G, Steffensen I-L, Tlustos C, Van Loveren H, Vernis L, Zorn H, Roos Y, Andryszkiewicz M, Liu Y and Chesson A, 2022. Scientific Opinion on the safety evaluation of the food enzyme phytepsin from *Cynara cardunculus* L. EFSA Journal 2022;20(12):7681, 9 pp. <https://doi.org/10.2903/j.efsa.2022.7681>

ISSN: 1831-4732

© 2022 Wiley-VCH Verlag GmbH & Co. KGaA on behalf of the European Food Safety Authority.

This is an open access article under the terms of the [Creative Commons Attribution-NoDerivs](https://creativecommons.org/licenses/by-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited and no modifications or adaptations are made.



The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.



Table of contents

Abstract.....	1
1. Introduction.....	4
1.1. Background and terms of reference as provided by the requestor.....	4
1.1.1. Background as provided by the European Commission.....	4
1.1.2. Terms of reference.....	5
1.2. Interpretation of the terms of reference.....	5
2. Data and methodologies.....	5
2.1. Data.....	5
2.2. Methodologies.....	5
3. Assessment.....	5
3.1. Source of the food enzyme.....	5
3.2. Production of the food enzyme.....	6
3.3. Characteristics of the food enzyme.....	6
3.3.1. Properties of the food enzyme.....	6
3.3.2. Chemical parameters.....	6
3.3.3. Purity.....	7
3.4. Toxicological data.....	7
3.4.1. Allergenicity.....	7
3.5. Dietary exposure.....	8
3.5.1. Intended use of the food enzyme.....	8
3.5.2. Dietary exposure estimation.....	8
3.6. Margin of exposure.....	8
4. Conclusions.....	8
5. Documentation as provided to EFSA.....	8
References.....	9
Abbreviations.....	9

1. Introduction

Article 3 of the Regulation (EC) No 1332/2008¹ provides definition for 'food enzyme' and 'food enzyme preparation'.

'Food enzyme' means a product obtained from plants, animals or 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 on food enzymes came into force. This Regulation applies to enzymes that are added to food to perform a technological function in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food, including enzymes used as processing aids. Regulation (EC) No 1331/2008² established the European Union (EU) procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

- it does not pose a safety concern to the health of the consumer at the level of use proposed;
- there is a reasonable technological need;
- its use does not mislead the consumer.

All food enzymes currently on the European Union market and intended to remain on that market, as well as all new food enzymes, shall be subjected to a safety evaluation by the European Food Safety Authority (EFSA) and approval via an EU Community list.

The 'Guidance on submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) lays down the administrative, technical and toxicological data required.

1.1. Background and terms of reference as provided by the requestor

1.1.1. Background as provided by the European Commission

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

Five applications have been introduced by the companies "Productos Nievi, SA" for the authorisation of the food enzyme rennet consisting of chymosin and pepsin from stomachs of young calves and sheep, "Avances Bioquímicos Alimentación, SL" for the authorisation of the food enzyme plant coagulant from the flowers of *Cynara cardunculus*, "Mitsubishi-Kagaku Foods Corporation" and "Kikkoman Biochemifa Company" for the authorisation of the food enzyme Tannase from *Aspergillus oryzae* (strains NBRC 110971 and 11-5, respectively) and from "Danisco US Inc." for the authorisation of the food enzymes Alpha-amylase from *Aspergillus niger* (DP-Azb60) and Catalase from a genetically modified strain of *Aspergillus niger* (DP-Azw58).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011³ implementing Regulation (EC) No 1331/2008, the Commission has verified that the application falls within the scope of the food enzyme Regulation and contain all the elements required under Chapter II of that Regulation.

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

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

³ Commission Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 64, 11.03.2011, pp. 15–24.

1.1.2. Terms of reference

The European Commission requests the European Food Safety Authority to carry out the safety assessment on the food enzymes rennet consisting of chymosin and pepsin from stomachs of young calves and sheep, plant coagulant from the flowers of *Cynara cardunculus*, Tannase from *Aspergillus oryzae* (strains NBRC 110971 and 11–5), Alpha-amylase from *Aspergillus niger* (DP-Azb60) and Catalase from a genetically modified strain of *Aspergillus niger* (Azw58) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. Interpretation of the terms of reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of the food enzyme plant coagulant from the flowers of *Cynara cardunculus*.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme vegetable coagulant from the flowers of *Cynara cardunculus*. The dossier was updated on 2 March 2015.

Additional information was requested from the applicant during the assessment process on 25 May 2015 and on 27 June 2022, and was subsequently provided.

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA 'Guidance on transparency in the scientific aspects of risk assessment' (EFSA, 2009b) and following the relevant guidance documents of the EFSA Scientific Committee.

The current 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) have been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance with the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021).

3. Assessment

The term phytepsin covers a number of aspartic endopeptidases present in cardoon.

IUBMB nomenclature	Phytepsin
Systematic name	–
Synonyms	–
IUBMB No	3.4.23.40
CAS No	219715-98-7
EINECS No	–

Phytepsins are used as coagulating agents leading to curdling of milk components. The enzyme is intended to be used in milk processing for cheese production.

3.1. Source of the food enzyme

The cardoon extract containing the food enzyme phytepsin is obtained from the pistils of dried flowers of *Cynara cardunculus* L. (cardoon).

Cynara cardunculus L. is a species belonging to the Asteraceae family. It is a herbaceous perennial plant native to an area in Southern Europe and Northern Africa around the Mediterranean. It is cultivated in Spain, Portugal and France in several different cultivar forms, including the cardoon and the globe artichoke, which are taller, less spiny and possess larger stems or flowers (Silva et al., 2022). The parts of the cardoon plant consumed by humans are the leaf stalks. The flower buds are also

employed as a vegetable rennet in cheesemaking in Spain and Portugal (Davila Fernández et al., 2010; Barbosa et al., 2020). No toxic effects have been reported for *Cynara cardunculus*.⁴

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

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004⁵, with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current good manufacturing practice.⁶

The food enzyme is obtained by extraction from the pistils of cardoon flowers. These are collected in the flowering season, dried at room temperature shielded from sunlight and conserved throughout the year to be used in cheesemaking until the next harvest. When required, [REDACTED] is added to the dried pistils and macerated [REDACTED]. The macerated product is then transferred to cloth, bags and [REDACTED] pressed. The crude filtrate obtained containing the food enzyme is concentrated by ultrafiltration and filter sterilised before packaging.⁷ The applicant provided information on the identity of the raw materials and substances involved in the extraction process of the food enzyme.⁸

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

A number of aspartic endopeptidases from cardoon have been described in the literature as dimeric proteins with molecular masses ranging from 14 to 35.5 kDa (Heimgartner et al., 1990; Cordeiro et al., 1994; Veríssimo et al., 1996; Faro et al., 1999; Sarmiento et al., 2009).

The in-house determination of the milk clotting activity of phytapsin is based on the clotting of reconstituted milk. The food enzyme is added to reconstituted milk powder (pH 6.5, 30°C) and the clotting time is determined visually. The enzyme activity is expressed in rennet unit/g (RU/g). One rennet unit (RU) is defined as the amount of protein that coagulates 10 mL of reconstituted milk at 30°C in 100 s.⁹

The food enzyme under assessment has a temperature optimum around 75°C (pH 6.5) and a pH optimum around pH 5.5 (30°C).¹⁰ Thermostability was tested after a pre-incubation of the food enzyme at 45°C for different periods (pH 5.5). Enzyme activity decreased by 60% within the first 10 h of incubation, showing no residual activity after 48 h.¹⁰

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (Table 1).¹¹ The mean total organic solids (TOS) of the three food enzyme batches for commercialisation is 2.7% and the mean enzyme activity/TOS ratio is 4.5 RU/mg TOS.

⁴ <https://www.gardenersworld.com/plants/cynara-cardunculus/>

⁵ 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.

⁶ Technical dossier/2nd submission/pp. 2, 22.

⁷ Technical dossier/2nd submission/pp. 15–18.

⁸ Technical dossier/2nd submission/pp. Annex IV.

⁹ Technical dossier/2nd submission/p. 46 and Additional Data/pp. 9–10/Additional data October 2022.

¹⁰ Technical dossier/2nd submission/Additional Data/pp. 10–12.

¹¹ Technical dossier/2nd submission/Additional Data/pp. 1–7.

Table 1: Composition of the food enzyme

Parameters	Unit	Batches		
		1	2	3
Phyepsin activity	RU/g ^(a)	120	120	120
Protein	%	< 2.0	< 2.0	< 2.0
Ash	%	9.2	9.1	9.1
Water	%	88.4	88.3	87.8
Total organic solids (TOS)^(b)	%	2.4	2.6	3.1
Phyepsin activity/TOS	UC/mg TOS	5.0	4.6	3.9

(a): RU/g: Rennet Unit/g (see Section 3.3.1).

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

3.3.3. Purity

The lead content in the three commercial batches was below 0.01 mg/kg¹¹ which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).^{12,13}

The food enzyme complies with the microbiological criteria (for total coliforms, *Enterobacteriaceae* and *Salmonella*),¹⁴ as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). Microbiological counts for *Staphylococcus* and *Listeria monocytogenes* were less than 10 colony forming units/g and absent in 25 g of sample, respectively.¹⁵

Residue data for an extensive list of pesticides were provided for one batch of food enzyme.¹⁵ The presence of aflatoxins (B1, B2, G1, G2), ochratoxin A, zearalenone, deoxynivalenol, toxin HT2, toxin T2 and fumonisins (B1, B2) was also examined in the same food enzyme batch. The Panel accepted the data on the one batch as it would be representative of the seasonal production of the pistils. All were below the limits of quantification (LoQ) of the applied analytical methods.^{15,16}

The Panel considered that the information provided on the purity of the food enzyme is sufficient and did not raise safety concerns.

3.4. Toxicological data

According to the Commission Implementing Regulation (EU) No 562/2012, 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 a plant 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 CEP Panel, 2021).

The Panel considers that these requirements are fulfilled, because:

- i) *C. cardunculus* L. leaf stalks are commonly consumed throughout Europe in raw or cooked form. The flower buds have been traditionally used for centuries as a vegetable rennet in the production of various cheeses in Spain and Portugal.
- ii) the manufacturing process of the food enzyme is not considered to introduce substances that could raise safety concerns. Contaminants and pesticides residues that could be carried over from the flowers of *C. cardunculus* L. were analysed and raised no issues.

3.4.1. Allergenicity

The potential allergenicity of the phytepsin produced with the non-genetically modified *Cynara cardunculus* L. was not assessed by comparing its amino acid sequence with those of known

¹² Technical dossier/2nd submission/p. 10.

¹³ LoD: Pb = 0.01 mg/kg.

¹⁴ Technical dossier/2nd submission/p. 10/Additional data October 2022.

¹⁵ Additional data October 2022.

¹⁶ LoQs: aflatoxins (B1, B2, G1, G2) = 1 µg/kg each; ochratoxin A = 2 µg/kg; zearalenone = 30 µg/kg; deoxynivalenol = 150 µg/kg; HT2 toxin = 100 µg/kg; T2 toxin = 25 µg/kg; fumonisins (B1, B2) = 100 µg/kg each.

allergens.¹⁵ The Panel recognises that phytepsins is a general term covering many aspartate endopeptidases, for which reason the comparison of the amino acid sequence of the exhaustive number of endopeptidases with known allergens is impractical. Nevertheless, the Panel could reach a conclusion based on the following evidence below.

No information was available on oral and respiratory sensitisation or elicitation reactions of this phytepsin.

Reference to some confirmed allergic reactions caused by the *C. cardunculus* plant refers to the possible consumption of different parts of the plant, such as the stem, flowers or unripe inflorescences (artichokes) (Paulsen, 2016).

Considering the extraction and processing of the food enzyme, the phytepsin from *C. cardunculus* L. might contain traces of allergens from cardoon flowers. The Panel considered that allergic reactions to this phytepsin can therefore not be excluded in individuals allergic to this plant. However, the likelihood of allergic reaction to the phytepsin from *C. cardunculus* L. is expected not to exceed the likelihood of allergic reactions to cardoon. As prevalence of allergic reaction to these foods is low, the likelihood of such reaction to occur to the food enzyme is also considered low.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in cheese production in dairy processing at a recommended use level of 350 mL of liquid enzyme preparation/1,000 L milk,¹⁷ corresponding to 9.5 mg TOS/L milk. Phytepsin is used to make cheese from goat and ewe milk.¹⁸

Phytepsin is added to milk as a coagulant to separate milk into solid curd and liquid whey. The majority of the food enzyme TOS partitions into the whey and is removed during the draining of the whey. Only a small portion of the food enzyme TOS remains in the curd (approximately 10%). The remaining rennet contributes to the ripening of cheese due to its proteolytic activity.

Based on data provided on thermostability (see Section 3.3.1), it is expected that the enzyme may remain active in cheese, depending on the respective cheesemaking process.

3.5.2. Dietary exposure estimation

The technology of extracting enzymes from cardoon and the technology of using the extract for cheesemaking have been for millennia, which remains the major source of human exposure to the food enzyme. Cheese and by-products of cheesemaking have been consumed by human in Europe and many other parts of the world for millennia. In addition, cardoon is consumed in some European countries, although this constitutes only a minor fraction of the overall exposure to the food enzyme in the EU.

In the view of the Panel, dietary exposure estimation was not required.

3.6. Margin of exposure

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

4. Conclusions

Based on the data provided and the origin of the food enzyme, the Panel considered that the food enzyme phytepsin extracted from the pistils of *Cynara cardunculus* L. does not raise safety concerns under the intended conditions of use.

5. Documentation as provided to EFSA

Vegetable Coagulant extracted from the cardoon flowers of *Cynara cardunculus*. March 2015. Submitted by AVANCES BIOQUÍMICOS ALIMENTACIÓN, S.L. (ABIASA). The dossier was updated on 2 March 2015.

¹⁷ Technical dossier/p. 24.

¹⁸ Technical dossier/p. 21.

Additional information. May 2015. Submitted by AVANCES BIOQUÍMICOS ALIMENTACIÓN, S.L. (ABIASA).

Additional information. October 2022. Submitted by AVANCES BIOQUÍMICOS ALIMENTACIÓN, S.L. (ABIASA).

References

- Barbosa CH, Andrade MA, Vilarinho F, Castanheira I, Fernando AL, Loizzo MR and Silva AS, 2020. A new insight on cardoon: exploring new uses besides cheese making with a view to zero waste. *Foods*, 9, 564. <https://doi.org/10.3390/foods9050564>
- Cordeiro MC, Salomé M and Brodelius PE, 1994. Tissue-specific expression of multiple forms of cyprosin (aspartic proteinase) in flowers of *Cynara cardunculus*. *Physiologia Plantarum*, 92, 645–653.
- Davila Fernández G, Zapatero L, Bartolomé B, Fuentes V and Alonso E, 2010. Cardoon allergy. *Allergologia et Immunopathologia*, 38, 165–166. <https://doi.org/10.1016/j.aller.2009.09.003>
- EFSA (European Food Safety Authority), 2009a. Guidance of EFSA prepared by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. *EFSA Journal* 2009;7(8):1305, 26 pp. <https://doi.org/10.2903/j.efsa.2009.1305>
- EFSA (European Food Safety Authority), 2009b. Guidance of the Scientific Committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: general principles. *EFSA Journal* 2009;7(5):1051, 22 pp. <https://doi.org/10.2903/j.efsa.2009.1051>
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré C, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mengelers M, Mortensen A, Rivière G, Steffensen I-L, Tlustos C, Van Loveren H, Vernis L, Zorn H, Glandorf B, Herman L, Aguilera J, Andryszkiewicz M, Gomes A, Kovalkovicova N, Liu Y, Rainieri S and Chesson A, 2021. Scientific Guidance for the submission of dossiers on Food Enzymes. *EFSA Journal* 2021;19(10):6851, 37 pp. <https://doi.org/10.2903/j.efsa.2021.6851>
- FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization), 2006. General specifications and considerations for enzyme preparations used in food processing in Compendium of food additive specifications. 67th Meeting FAO JECFA Monographs 3, 63–67. Available online: <http://www.fao.org/3/a-a0675e.pdf>
- Faro C, Ramalho-Santos M, Vieira M, Mendes A, Simoes I, Andrade R, Veríssimo P, Lin XL, Tang J and Pires E, 1999. Cloning and characterization of cDNA encoding cardosin A, an RGD-containing plant aspartic proteinase. *The Journal of Biological Chemistry*, 274, 28724–28729.
- Heimgartner U, Pietrzak M, Geertsen R, Brodelius P, DaFigueiredo AC and Pais MSS, 1990. Purification and partial characterization of milk clotting proteases from flowers of *Cynara cardunculus*. *Phytochemistry*, 29, 1405–1410.
- Paulsen E, 2016. Systemic allergic dermatitis caused by sesquiterpene lactones. *Contact Dermatitis*, 76, 1–10. <https://doi.org/10.1111/cod.12671>
- Sarmento AC, Lopes H, Oliveira CS, Vitorino R, Samyn B, Sergeant K, Debyser G, Van Beeumen J, Domingues P, Amado F, Pires E, Domingues RM and Barros MT, 2009. Multiplicity of aspartic proteinases from *Cynara cardunculus* L. *Planta*, 230, 429–439.
- Silva LR, Jacinto TA and Coutinho P, 2022. Bioactive compounds from cardoon as health promoters in metabolic disorders. *Foods*, 11(3), 336. <https://doi.org/10.3390/foods11030336>
- Veríssimo P, Faro C, Moir AJG, Lin Y, Tang J and Pires E, 1996. Purification, characterization and partial amino acid sequencing of two new aspartic proteinases from fresh flowers of *Cynara cardunculus* L. *European Journal of Biochemistry*, 235, 762–768.

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 Organisation of the United Nations
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LoQ	limit of quantification
RU	rennet unit
TOS	total organic solids
WHO	World Health Organization