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

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Abstract

The food enzyme phytepsin (EC 3.4.23.40) is extracted from the pistils of the cardoon (*Cynara cardunculus* L.) by seven manufacturers represented by the Regulation Council of Protected Designation of Origin Torta del Casar. 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. A search for similarity of the amino acid sequences of the food enzyme to known allergens was made and no matches were found. 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.

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

Requestor: European Commission

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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 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 1331/2008[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 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 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 on food enzymes.

An application has been introduced by the Faculty of Veterinary Sciences of the University of Extremadura for the authorisation of a food enzyme obtained from the Cardoon flowers (*Cynara cardunculus* var. sylvestris).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] 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.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessment on the food enzyme obtained from the Cardoon flowers (*Cynara cardunculus* var. sylvestris) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 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.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme obtained from Cardoon flowers (*Cynara cardunculus* var. sylvestris). The dossier was submitted on 10 March 2015.

Additional information was requested from the applicant during the assessment process on 16 May 2022, 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, 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) has been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance with the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021).

3. Assessment

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	219,715–98-7
EINECS No	–

Phytepsins are used as a coagulating agent 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 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 Fernàndez 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 852/2004,[5] 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. Dried pistils are submerged in drinking

[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] Additional data August 2022.

water for a few hours and then macerated. After filtration through a cloth, the filtrate containing the food enzyme is directly employed in cheesemaking as a coagulating agent.[7]

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 (Veríssimo et al., 1996; Faro et al., 1999; Sarmento et al., 2009; Heimgartner et al., 1990; Cordeiro et al., 1994).

The in-house determination of phytepsin activity is based on the hydrolysis of casein (reaction conditions: pH 7.5, 37°C, 30 min). The enzymatic activity is determined by measuring the reaction of the released tyrosine with Folin–Ciocalteau's reagent spectrophotometrically at 660 nm. It is expressed in Unit/g. One Unit is defined as the amount of the enzyme that releases one μmol of tyrosine equivalent.[8]

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

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for four samples from each of the seven different cheese manufacturers (Table 1).[9]

Table 1: Composition of the food enzyme

Parameters	Unit	Batches by seven manufacturers: range of four samples						
		1	2	3	4	5	6	7
Phytepsin activity	Unit/mL[(a)]	0.35–0.42	0.27–0.36	0.32–0.37	0.29–0.47	0.36–0.43	0–37-0.40	0.30–0.39
Ash	%	0	0	0	0	0	0	0
Water	%	98.2–99.7	97.4–99.7	98.2–99.8	98.0–99.7	97.6–99.6	98.9–99.8	97.4–99.7
Total organic solids (TOS)[(b)]	%	0.3–1.8	0.3–2.6	0.2–1.8	0.3–2.0	0.4–2.3	0.2–1.1	0.3–2.6
Phytepsin activity/TOS	UNIT/mg TOS	NP[(c)]	NP	NP	NP	NP	NP	NP

(a): UNIT: U/g (see Section 3.3.1).
(b): TOS calculated as 100% – % water – % ash.
(c): NP: not provided.

3.3.3. Purity

The lead content in three samples was below 5 mg/kg,[9] which complied with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[10,11]

The food enzyme complied with the microbiological criteria (for total coliforms, *Escherichia coli* and *Salmonella*)[9] as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[10]

[7] Technical dossier/3rd submission/pp. 29-30/Additional data August 2022.
[8] Technical dossier/2nd submission/pp. 14–15.
[9] Technical dossier/2nd submission/SiPA report 2017/pp. 3–6.
[10] Technical dossier/2nd submission/p. 11/Additional data August 2022.
[11] LoQ: Pb = 0.5 mg/L.

The presence of ochratoxin A, aflatoxins (B1, B2, G1, G2), zearalenone, diacetoxyscirpenol (DAS), deoxynivalenol (DON), HT2 toxin, T2 toxin and nivalenol was examined in three food enzyme batches and all were below the limits of quantification (LoQ) of the applied analytical methods.[12,13]

Residue data for an extensive list of pesticides were provided for three samples from the seven cheese manufacturers.

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 CEF Panel, 2009).

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 pesticide 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 *C. cardunculus* L. was assessed by comparing the published amino acid sequences 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, no matches with known allergens were found.[14]

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

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 milk processing for cheese production at a recommended use level of 50–230 g dried pistils/100 l milk.[15] Phytepsin is generally used to make cheese from ewe milk.[16]

[12] LoQs: aflatoxins (B1, B2, G1, G2) = 0.01 mg/kg each; ochratoxin A = 0.001 mg/kg, zearalenone, diacetoxyscirpenol (DAS), deoxynivalenol (DON), HT2 toxin, T2 toxin and nivalenol = 0.01 mg/kg each.
[13] Technical dossier/2nd submission/SiPA Report 2015/pp. 1–3.
[14] Additional data August 2020.
[15] Technical dossier/Section 3.4-iv.
[16] Technical dossier/Section 3.4-iii.

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 mostly 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 general proteolytic activity.

Based on data provided on thermostability (see Section 3.3.1), it is expected that the enzyme remains active in cheese.

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. Conclusion

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

Aqueous extract from *Cynara cardunclus L*. var. sylvestris (Lamk) for milk coagulation in "Torta del Casar" cheese. March 2015. Submitted by Faculty of Veterinary Science of the University of Extremadura. The dossier was updated on 10 March 2015.

Additional information. January 2018. Submitted by Faculty of Veterinary Science of the University of Extremadura.

Additional information. August 2022. Submitted by Faculty of Veterinary Science of the University of Extremadura.

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(3), 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

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010. Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal 2010;8(7):1700, 168 pp. https://doi.org/10.2903/j.efsa.2010.1700

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, pp. 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, 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
DAS	diacetoxyscirpenol
DON	deoxynivalenol
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
LoQ	limit of quantification
WHO	World Health Organization