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## 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 cardoon (*Cynara cardunculus* L.) by QUALIFICA/origIn PORTUGAL. 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.

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**Correspondence:** [fip@efsa.europa.eu](mailto: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

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## 1. Introduction

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

'Food enzyme' means a product obtained from plants, animals or microorganisms 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 on food enzymes came into force. This Regulation applies to enzymes that are added to food to perform a technological function in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food, including enzymes used as processing aids. Regulation (EC) No 1331/2008<sup>2</sup> established the European Union (EU) procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

- It does not pose a safety concern to the health of the consumer at the level of use proposed;
- There is a reasonable technological need;
- Its use does not mislead the consumer.

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

The 'Guidance on submission of a dossier on food enzymes for safety evaluation' (EFSA, 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.

Five applications have been introduced by the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) for the authorisation of the Food Enzyme Papain from *Carica papaya*, and the companies "Qualifica" for the authorisation of the food enzyme Plant coagulant from Cardoon flower (*Cynara cardunculus* L.), "Advanced Enzyme Technologies Ltd." for the authorisation of the food enzyme Triacylglycerol lipase from a genetically modified strain of *Aspergillus niger* agg. (strain FL105SC); "Kerry Ingredients & Flavours" for the authorisation of the food enzyme Endo-1,3(4)-beta-glucanase from a genetically modified strain of *Bacillus subtilis* (strain CBS 613.94) and "AB Enzymes GmbH." for the authorisation of the food enzyme Endo-1,4-beta-glucanase from a genetically modified strain of *Trichoderma reesei* (RF5261).

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

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

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

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

### 1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes Papain from *Carica papaya*, Plant coagulant from Cardoon flower (*Cynara cardunculus* L.), Triacylglycerol lipase from a genetically modified strain of *Aspergillus niger* agg. (strain FL105SC); Endo-1,3(4)-beta-glucanase from a genetically modified strain of *Trichoderma reesei* (RF5261) 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 Cardoon flower (*Cynara cardunculus* L.)

## 2. Data and methodologies

### 2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme plant coagulant from the Cardoon flower (*Cynara cardunculus* L.). The dossier was updated on 24 February 2015 and 31 January 2016.

Additional information was requested from the applicant during the assessment process on 27 November 2015 and 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	219715-98-7
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 *Cynara cardunculus* L. (cardoon).<sup>4</sup>

*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

<sup>4</sup> Technical dossier/Additional data August 2022.

vegetable rennet in cheese making in Spain and Portugal (Davila Fernández et al., 2010; Barbosa et al., 2020). No toxic effects have been reported for *Cynara cardunculus*.<sup>5</sup>

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

### 3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004<sup>6</sup>, with food safety procedures based on hazard analysis and critical control points, and in accordance with current good manufacturing practice.<sup>4</sup>

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 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.<sup>7</sup>

The Panel considered that sufficient information has been provided on the manufacturing process and the quality assurance system implemented by the applicant to exclude issues of concern.

### 3.3. Characteristics of the food enzyme

#### 3.3.1. Properties of the food enzyme

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; Verissimo et al., 1996; Faro et al., 1999; Sarmiento et al., 2009).

The determination of phytepsin activity is based on the official method ISO 11815|International Dairy Federation (IDF) standard 157:2007. The time needed for visual flocculation of a standard milk substrate prepared with a calcium chloride solution of 0.5 g per litre (pH  $\approx$  6.5) is determined. The clotting time of a phytepsin sample is compared to that of a reference standard with known milk-clotting activity, determined using the ISO 15163|IDF standard 110:2012. The total milk-clotting activity is expressed in International Milk-Clotting Units (IMCU).<sup>8</sup>

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). Thermostability was tested after a pre-incubation of the food enzyme at 45°C at different times (pH 5.5). Enzyme activity decreased by 60% within the first 10 h of incubation, showing no residual activity after 48 h.<sup>9</sup>

#### 3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for 48 batches (Table 1).<sup>10</sup>

**Table 1:** Compositional data from 48 batches of the food enzyme

Parameter	Unit	Mean	Minimum–maximum
Phytepsin	IMCU/g batch <sup>(a)</sup>	111	85.0–135
Protein	%	12.2	10.8–14.1
Ash	%	4.4	3.7–5.5
Water	%	6.7	6.2–7.6
TOS <sup>(b)</sup>	%	88.9	NP
Phytepsin activity/TOS	U/mg TOS	0.1	NP <sup>(c)</sup>

(a): IMCU/g: (see Section 3.3.1).

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

(c): NP: not provided.

<sup>5</sup> <https://www.gardenersworld.com/plants/cynara-cardunculus>.

<sup>6</sup> 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.

<sup>7</sup> Technical dossier/3rd submission/pp. 29–30/Additional data August 2022.

<sup>8</sup> Technical dossier/3rd submission/pp. 22–23/Doc n16 FIL IDF 2007.

<sup>9</sup> Technical dossier/3rd submission/pp. 23–26.

<sup>10</sup> Technical dossier/3rd submission/p. 21/Additional data July 2016.

### 3.3.3. Purity

The lead content in three batches was below 5 mg/kg, which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).<sup>11,12</sup>

The food enzyme complied with the microbiological criteria (for *Escherichia coli* and *Salmonella*), as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).<sup>13</sup> Microbiological counts for *Staphylococcus* and *Listeria monocytogenes* were less than 10 colony forming units/g and absent in 25 g of sample, respectively.

Residue data for an extensive list of pesticides were provided for three batches of the food enzymes.<sup>14</sup>

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<sup>15</sup>, an application for the safety evaluation of a food enzyme does not need to include toxicological data if the food enzyme is obtained from edible parts of 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 cheese 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 not assessed by comparing its amino acid sequence with those of known allergens.<sup>16</sup> The Panel recognises that phytepsins is a general term covering many different 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.

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.

<sup>11</sup> Additional data August 2022/Appendix C/BA Res1.

<sup>12</sup> LoD: Pb = 0.474 mg/L.

<sup>13</sup> Additional data August 2022/Appendix B/BA M Flor/BA M Infusao.

<sup>14</sup> Additional data July 2016; Additional data August/Appendix C/BA Res2/Boletim de Analise.

<sup>15</sup> Commission Implementing Regulation (EU) No 562/2012 of 27 June 2012 amending Commission Regulation (EU) No 234/2011 with regard to specific data required for risk assessment of food enzymes. OJ L 168, 28.6.2012, pp. 21–23.

<sup>16</sup> Additional data August 2022.



### 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 0.2–0.6 g dried pistils/L of milk.<sup>17</sup> Phytepsin is generally used to make cheese from ewe milk.<sup>18</sup>

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 cheese making 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

Cardoon flower extract for human consumption. February 2015. Submitted by QUALIFICA Origin. The dossier was updated on 31 January 2016.

Additional information. November 2015. Submitted by QUALIFICA Origin.

Additional information. August 2022. Submitted by QUALIFICA Origin.

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<sup>17</sup> Additional information August 2022/p. 3.

<sup>18</sup> Technical dossier/p. 27 and p. 34.



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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 Organisation of the United Nations
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
IMCU	International Milk-Clotting Units
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LoD	limit of detection
TOS	total organic solids
WHO	World Health Organisation