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Safety evaluation of the food enzyme catalase from porcine liver

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Abstract

The food enzyme catalase (EC 1.11.1.6) is obtained from porcine liver by Laboratorios Arroyo S.A. It is intended to be used in a broad range of food processes. The Panel noted that the manufacturing process involved the use of a solvent not permitted in the production of food ingredients which include food enzymes. In addition, the evidence provided showed that the manufacturing process could not be guaranteed to inactivate viruses originating from the starting material, including the human zoonotic pathogen Hepatitis E virus. Consequently, the Panel concluded that the use of catalase extracted from porcine liver may present a health risk.

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† Deceased.

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.....	4
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.....	5
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.....	9
4. Conclusions.....	9
5. Documentation as provided to EFSA.....	9
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 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 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 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 applicant "Laboratorios Arroyo S.A." for the authorisation of the food enzyme: Catalase from hog (pig) liver.

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 contains all the elements required under Chapter II of that Regulation.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessment on the following food enzyme: Catalase from hog (pig) liver 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, 11.3.2011, 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 catalase from porcine liver.

Additional information was requested from the applicant during the assessment process on 12 February 2020 and on 08 June 2021 (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 existing guidance documents of EFSA Scientific Committees.

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 to the methodology described in the CEF Panel 'Statement on the exposure assessment of food enzymes' (EFSA CEF Panel, 2016).

3. Assessment

IUBMB nomenclature	Catalase
Systematic name	Hydrogen-peroxide:hydrogen-peroxide oxidoreductase
Synonyms	Caperase
IUBMB No	EC 1.11.1.6
CAS No	9001-05-2
EINECS No	232-577-1

Catalase catalyses the decomposition of hydrogen peroxide to water and oxygen. It is intended to be used in several food processes, e.g. cheese production, bleaching of whey, egg processing, as a bleaching agent in emulsifiers containing fatty acid esters, in tripe, beef, herring and instant tea, and removal of SO₂ in starch processing and wine vinegar.

3.1. Source of the food enzyme

The food enzyme is produced from the liver of pigs (*Sus scrofa domestica*). The food enzyme is exclusively obtained from animals slaughtered and approved for human consumption, free of notifiable diseases (i.e. African Swine Fever (ASF), Classical Swine Fever (CSF), Food and Mouth Disease (FMD) and Swine Vesicular Disease (SVD)). Verification is performed by veterinarians in charge of the registered establishments for the slaughtering.⁴ Pigs are not included in the list of the specific risk material defined by Commission Regulation (EU) 2015/1162⁵. Porcine liver is an edible offal as defined in Regulation (EC) No 853/2004⁶. It is commonly consumed in the European Union. The porcine livers are collected following the requirements of the relevant EU hygiene regulations.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004⁷, with food safety procedures based on hazard analysis and critical control points, and in accordance with current good manufacturing practice.⁸

⁴ Technical dossier/Document 8.

⁵ Commission Regulation (EU) No 2015/1162 of 15 July 2015 amending Annex V to Regulation (EC) No 999/2001 of the European Parliament and of the Council laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies.

⁶ Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs. OJ L139/55, 30.4.2004.

⁷ 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.

⁸ Technical dossier/Additional information March 2021/Document 1.

The food enzyme is extracted from porcine liver. [REDACTED]

[REDACTED] The applicant provided information on the identity of the substances used in the extraction and in the subsequent processing of the food enzyme.⁹

The use of the solvent [REDACTED] for food ingredients production is not permitted under European legislation (Directive 2009/32/EC¹⁰).

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

Catalase is a tetramer of four polypeptide chains of [REDACTED] amino acids each. The molecular mass of each polypeptide was calculated to be [REDACTED] kDa ([REDACTED]).¹¹ The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis. A consistent protein pattern was observed across all batches.¹² No other enzyme activities were reported.

The in-house determination of catalase activity is based on decomposition of hydrogen peroxide. The sample is mixed with a hydrogen peroxide solution (25°C). The resulting solution is acidified with sulfuric acid. Potassium iodide is added, which reacts with the residual hydrogen peroxide to release iodine. The enzymatic activity is determined by titrating the released iodine with sodium thiosulfate. It is expressed in Baker Units (BU)/g. One BU is defined as the amount of catalase that decomposes 266 mg of hydrogen peroxide under the conditions of the assay.¹³

The food enzyme has a temperature optimum at around 37°C (phosphate buffer pH 7.0) and a pH optimum at around pH 5.8 (25°C). The enzyme activity decreased above 45°C, showing no residual activity above 60°C.^{14,15}

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches of the liquid food enzyme preparation (Table 1).¹⁶ The mean total organic solids (TOS) is 22.1% and the mean enzyme activity/TOS ratio is about 0.8 BU/mg TOS.

Table 1: Composition of the liquid food enzyme preparation

Parameter	Unit	Batches		
		1	2	3
Catalase activity	BU/g batch ^(a)	175	158	218
Protein	%	6.5	6.8	5.5
Ash	%	3.7	3.7	4.0
Water	%	74.9	74.2	73.3
Total organic solids (TOS) ^(b)	%	21.4	22.1	22.7
Activity/mg TOS	BU/mg TOS	0.8	0.7	1.0

(a): BU: Baker units (see Section 3.3.1).

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

⁹ Technical dossier/p. 12–14 and Document 7.

¹⁰ Directive 2009/32/EC of the European Parliament and of the Council of 23 April 2009 on the approximation of the laws of the Member States on extraction solvents used in the production of foodstuffs and food ingredients.

¹¹ Technical dossier/p. 6–7.

¹² Technical dossier/Additional information March 2021/Document 3.

¹³ Technical dossier/Document 9.

¹⁴ Technical dossier/Documents Catalase 2017/Document D.

¹⁵ Technical dossier/Additional information March 2021/Document 4.

¹⁶ Technical dossier/ Documents Catalase 2017/Documents A, B, C and G.

3.3.3. Purity

The lead content in the three commercial batches was below 0.50 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 mercury arsenic and cadmium were below the respective limit of quantification (LoQ) of the employed methodologies.¹⁷

Residual amounts of [REDACTED] were detected in the three (liquid) batches analysed (mean = 176 $\mu\text{g/L}$; range 8.6–311 $\mu\text{g/L}$).¹⁸

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). Additionally, aerobic bacteria, yeasts and filamentous fungi, *Enterobacteriaceae*, *Staphylococcus aureus*, *Clostridium* spp. and *Listeria* were tested, and their levels did not raise safety concern.¹⁹

Three batches of the food enzyme were tested by RT-PCR for the presence of Parvovirus, Circovirus, Norovirus and Hepatitis E virus (HEV). All three batches were positive for porcine Parvovirus and Circovirus and, more importantly, one of the three batches was positive for HEV. This data indicates that the manufacturing process may not guarantee the inactivation of viruses, including the human zoonotic pathogen HEV. This may represent a hazard.

3.4. Toxicological data

According to the Commission Implementing Regulation (EU) No 562/2012²⁰, 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, 2009a).

The Panel considers that these requirements are fulfilled, because:

- i) porcine liver is consumed throughout the world as a meat product, including European countries (see e.g., Toldrà et al., 2012);
- ii) the manufacturing process of the food enzyme is not considered to introduce substances that could raise safety concerns, apart from [REDACTED]. The compliance with meat inspection requirements and good hygienic practice is met, as all porcine livers used in the manufacturing of the catalase are sourced from plants registered with the regulatory bodies of the originating countries and comply to European health standards. Veterinary Certificates are issued for each batch of product, which state that the livers are sourced from government-inspected animals, are certified safe by an authorised veterinarian in the country of origin and are covered by a Food Safety and Inspection Program;
- iii) the compositional data provided on the food enzyme preparation are considered sufficient.

3.4.1. Allergenicity

The potential allergenicity of the food enzyme catalase derived from porcine liver was not assessed by comparing its amino acid sequence with those of known allergens.

No information is available on oral and respiratory sensitisation or elicitation reactions of this catalase.

Pig is not a source included in the list of substances or products causing allergies or intolerances (Regulation (EU) No 1169/2011²¹). There are no reports of allergy associated with consumption of

¹⁷ Technical dossier/Additional data March 2021/Annex 5.1. (LOQ provided for batches of 2020: As = 0.5 mg/kg; Cd = 0.2 mg/kg; Hg = 0.02 mg/kg; Pb = 0.5 mg/kg).

¹⁸ Technical dossier/Additional data September 2021/Document 2.

¹⁹ Technical dossier/Document 5 and Additional information February 2021/Document 5.

²⁰ 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.

²¹ 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.

pork liver or enzymes from pork liver. Respiratory allergic reactions to catalases from sources other than pig liver have been described (Green and Beezhold, 2011), but several studies have shown that adults sensitised to an enzyme through the respiratory tract 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). The Panel noted that an allergic reaction upon oral ingestion of this catalase from porcine liver in respiratorily sensitised individuals to catalase cannot be ruled out, but the likelihood of such a reaction to occur is considered to be low.

The Panel notes that lysozyme, a known allergen (Annex II of the Regulation (EU) No 1169/2011²²), is used during the downstream processing of the food enzyme and is likely to be present in the final product.

The Panel considers that the likelihood of food allergic reactions or intolerances to this food enzyme obtained from livers of pigs, in particular associated with lysozyme, cannot be excluded.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The applicant indicated a broad range of uses for this food enzyme. According to the applicant, it can be added to milk, egg or many other foods or food commodities to remove hydrogen peroxide or it can be added to grape juice to reduce SO₂ during wine and grape vinegar production.²³ The applicant also provided a long list of FoodEx categories for possible use of this food enzyme.²⁴

Using the 'EC working document describing the food processes in which food enzymes are intended to be used' as the reference, the Panel was able to translate some of this information into harmonised food manufacturing processes (see Table 2).

Table 2: Possible uses allocated to food processes by the Panel and recommended use levels of the food enzyme as proposed by the applicant

Food manufacturing process ^(a)	Raw material	Recommended dosage of the food enzyme ^(b)
Milk processing for cheese production and by-products	Milk	Up to 1 g enzyme/1,000 L milk ^(c)
Egg processing	Egg	165 U/kg ^(d)
Meat and fish processing	Tripe, beef feet, herring	Not specified
Wine and wine vinegar production	Grape, wine	Not specified
Grain treatment for the production of starch and gluten fractions	Starch slurry	Not specified
Tea processing	Tea	Not specified
Starch processing for syrup production	Syrup	Not specified
Fats and oil processing to produce emulsifier containing fatty acid esters	Emulsifiers	Not specified

(a): The description has been harmonised by EFSA according to the 'EC working document describing the food processes in which food enzymes are intended to be used' – not yet published at the time of adoption of this opinion.

(b): Based on bibliographic searches provided by the applicant.

(c): Technical dossier/Additional data September 2021.

(d): Technical dossier/p. 15.

3.5.2. Dietary exposure estimation

Although the applicant provided a list of potential applications of the food enzyme, specific use levels for most of the individual processes were not provided. Therefore, dietary exposure to the food enzyme-TOS could not be estimated.

²² 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.

²³ Technical dossier/Risk assessment data/p. 14–17 and Additional data March 2021/Document 7.

²⁴ Technical dossier/Additional data September 2021.

3.6. Margin of exposure

Since no toxicological assessment was considered necessary by the Panel and dietary exposure could not be estimated, the margin of exposure was not calculated.

4. Conclusions

The manufacturing process of the food enzyme involves the use of [REDACTED], a solvent not permitted in the production of food ingredients, which includes food enzymes.

The evidence provided showed that the manufacturing process could not be guaranteed to inactivate viruses originating from the starting material, including Hepatitis E virus, a known human pathogen.

The Panel notes that the food enzyme may contain egg white lysozyme, a known allergen.

Based on these data, the Panel concluded that the use of catalase extracted from porcine liver may present a health risk.

5. Documentation as provided to EFSA

- 1) Catalase from hog (pig) liver. March 2015. Submitted by Laboratorios Arroyo S.A.
- 2) Additional information. March 2021 and September 2021. Submitted by Laboratorios Arroyo S.A.

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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
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton

LoD limit of detection
RT-PCR real time polymerase chain reaction
SDS–PAGE sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS total organic solids
WHO World Health Organization