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Safety evaluation of the food enzyme catalase from porcine liver

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Abstract

The food enzyme catalase (hydrogen-peroxide:hydrogen-peroxide oxidoreductase; EC 1.11.1.6) is obtained from porcine liver by Avances Bioquímicos Alimentación, S.L. (Spain). The food enzyme is intended to be used in cheese production for decomposition of hydrogen peroxide in brine. The manufacturing process involves the use of a solvent not permitted in the production of foods and food ingredients according to Directive 2009/32/EC. Consequently, the food enzyme does not comply with the existing requirements in the EU.

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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 1332/2008¹ 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 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 1332/2008¹ on food enzymes.

Five applications have been introduced by the companies 'Danisco US Inc.' for the authorisation of the food enzyme cellulase from *Penicillium funiculosum* (strain DP-Lzc35), 'Advanced Enzyme Technologies Ltd.' for the authorisation of the food enzyme triacylglycerol lipase from a genetically modified strain of *Aspergillus niger agg* (strain FL108SC), 'Avances Bioquimicos Alimentación, S.L.' for the authorisation of the food enzyme catalase from porcine livers and 'Nagase (Europa) GmbH' for the authorisation of the food enzymes 1,4-alpha-glucan branching enzyme from *Geobacillus stearothermophilus* (strain TRBE14) and urease from *Lactobacillus fermentum* (strain 48/72).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011³ implementing Regulation (EC) No 1331/2008², 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.

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, p. 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 cellulase from *Penicillium funiculosum* (strain DP-Lzc35), triacylglycerol lipase from a genetically modified strain of *Aspergillus niger agg* (strain FL108SC), catalase from porcine livers and 1,4-alpha-glucan branching enzyme from *Geobacillus stearothermophilus* (strain TRBE14) and urease from *Lactobacillus fermentum* (strain 48/72) in accordance with Article 17.3 of Regulation (EC) No 1332/2008¹ 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 food enzyme catalase from porcine liver.

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 livers. The dossier was updated on 22 March 2016.

Additional information was requested from the applicant during the assessment process on 29 January 2020 and received on 18 January 2022 (see 'Documentation provided to EFSA').

Following the request for additional data sent by EFSA on 29 January 2020, the applicant requested a clarification teleconference on 22 June 2020, after which the applicant provided additional data on 18 January 2022.

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 the EFSA Scientific Committee.

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

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

Catalases catalyse the decomposition of hydrogen peroxide to water and oxygen. The food enzyme is intended to be used in cheese production for decomposition of hydrogen peroxide in brine.⁴

3.1. Source of the food enzyme

The food enzyme is a crude animal extract containing catalase activity which is produced from the liver of pigs (*Sus scrofa domesticus*).⁵

⁴ Technical dossier/Additional information, 18.1.2022.

⁵ Technical dossier/p. 52–75/Annex IV.

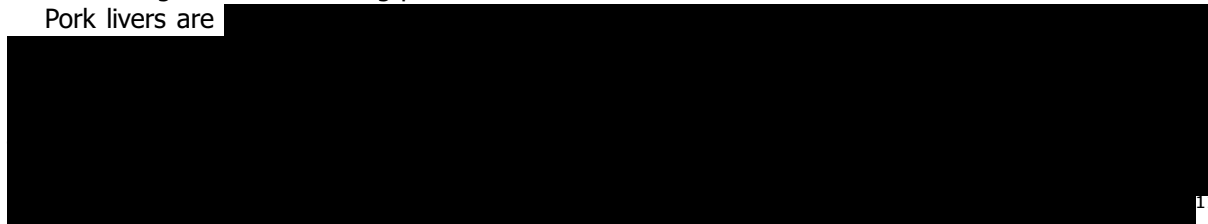
The food enzyme is exclusively obtained from healthy animals slaughtered under the supervision of official health authorities, following the requirements of the relevant EU hygiene regulations, the Food Hygiene Regulation (EC) No 852/2004⁶ and Regulation (EC) No 853/2004⁷.

Pigs are not included in the list of the specific risk material defined by Commission Regulation (EU) 2015/1162⁸. Porcine liver is an edible offal as defined in Regulation (EC) No 853/2004⁷. It is commonly consumed in the EU. The porcine livers are collected following the requirements of the relevant EU hygiene regulations.

3.2. Production of the food enzyme⁹

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004⁶, with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current good manufacturing practice.¹⁰

Pork livers are



The applicant provided information on the identity of the substances used in the extraction and in the subsequent downstream processing of the food enzyme.

The use of [redacted] as a solvent for foods and food ingredients is not permitted under European legislation (Directive 2009/32/EC¹²).

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme⁴

The catalase is a tetramer of four polypeptide chains of 527 amino acids each. The molecular mass of each monomer was calculated to be 59.9 kDa¹³ (Uniprot, O62839).

No other enzymatic activities were reported.¹⁴

The in-house determination of catalase activity¹⁵ is based on the decomposition of hydrogen peroxide. The decrease of absorbance is measured spectrophotometrically at 240 nm. The enzyme activity is expressed in catalase units (U)/g. One U is defined as the amount of enzyme that decomposes 1 μmol hydrogen peroxide in one minute at 25°C and pH 7.¹⁶

The food enzyme has a temperature optimum around [redacted]°C (pH [redacted]), and pH optimum of pH [redacted] ([redacted]°C).⁴ Thermostability was tested after a pre-incubation of the food enzyme for [redacted] min at different temperatures (pH [redacted]). Enzyme activity decreased above [redacted]°C and no residual activity was detected at [redacted]°C.¹⁷

⁶ 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, p. 1–54.

⁷ 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 L 139, 30.4.2004, p. 55–205.

⁸ 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. OJ L 188, 16.7.2015, p. 3–5.

⁹ Technical dossier/p. 16–21; Technical dossier/p. 52–81/Annex IV and Annex V.

¹⁰ Technical dossier/Manual ABIASA HACCP.

¹¹ Technical dossier/p. 17–20.

¹² 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. OJ L 141, 6.6.2009, p. 3–11.

¹³ Technical dossier/p. 31.

¹⁴ Technical dossier/2nd submission/5b Docs.

¹⁵ Technical dossier/p. 12; Technical dossier/p. 10/Figure 2; Technical dossier/p. 45–47/Annex II.

¹⁶ Technical dossier/p. 9–10; Technical dossier/p. 45–47/Annex II.

¹⁷ Technical dossier/p. 13–15.

3.3.2. Chemical parameters¹⁸

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (Table 1).¹⁴ The average total organic solids (TOS) of the three food enzyme batches is 17.5% and the average enzyme activity/mg TOS is 1,434 U/mg TOS.

Table 1: Compositional data of the food enzyme

Parameters	Unit	Batches		
		1	2	3
Catalase activity	U/mL batch ^(a)	250,000	250,000	250,000
Protein	%	2.51	2.34	2.47
Ash	%	1.30	0.99	1.30
Water	%	78.70	80.71	80.46
██████████ ⁴	%	1.32	1.62	1.18
Total organic solids (TOS)^(b)	%	18.68	16.68	17.06
Activity/mg TOS	U/mg TOS	1,338	1,499	1,465

(a): U: Unit (see Section 3.3.1).

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

3.3.3. Purity⁴

The lead content in the three commercial batches was below 0.01 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).¹⁹

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).⁴ No antimicrobial activity was detected in any of the tested batches.²⁰

The applicant stated that no measurable amounts of organic solvents used in extraction (██████████) remain in the final enzyme product.²¹ However, regardless of the residual amount, the use of ██████████ is not permitted as an extraction solvent according to Directive 2009/32/EC¹².

3.4. Toxicological data

As the manufacture process of the food enzyme is not compliant with European legislation, the Panel did not proceed with the evaluation of toxicity and allergenicity.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme²²

The food enzyme is added to brine during the salting of cheeses at the recommended use level of 0.35 mL/100 kg of brine⁴, corresponding to 0.61 mg TOS/kg brine. In cheese making, catalase is used to decompose hydrogen peroxide to water and oxygen. The applicant specified that this food enzyme will not be used for the production of infant and follow-on formula.⁴

3.5.2. Dietary exposure estimation

As the food enzyme does not comply with the existing requirements within the EU (see Section 3.3.3), the Panel did not estimate a dietary exposure to the food enzyme-TOS.

¹⁸ Technical dossier/p. 38–47/Annex I, Annex II; Technical dossier/2nd submission/5b Docs; Technical dossier/Additional information, 18.1.2022.

¹⁹ Technical dossier/Additional information, 18.1.2022: LOD: Lead = 0.01 mg/kg.

²⁰ Technical dossier/p. 38–47/Annex I and Annex II; Technical dossier/2nd submission/5b Docs.

²¹ Technical dossier/p. 8.

²² Technical dossier/p. 24–25; Technical dossier/Additional information, 18.1.2022.

4. Conclusions

The manufacturing process of the food enzyme involves the use of [REDACTED] a solvent not permitted in the production of foods and food ingredients according to Directive 2009/32/EC¹². Consequently, the food enzyme does not comply with the existing requirements in the EU.

5. Documentation as provided to EFSA

Technical dossier 'Catalase from porcine livers'. 4 February 2016 (1st submission) and 22 March 2016 (2nd submission). Submitted by Avances Bioquímicos Alimentación, S.L. (Spain).

Additional information. 18 January 2022. Submitted by Avances Bioquímicos Alimentación, S.L. (Spain).

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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
LOD	limit of detection
TOS	total organic solids
U	unit
WHO	World Health Organization