SCIENTIFIC OPINION

efsa JOURNAL

ADOPTED: 12 September 2023

doi: 10.2903/j.efsa.2023.8259

Safety evaluation of the food enzyme triacylglycerol lipase from the genetically modified *Aspergillus luchuensis* strain FL105SC

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, Lieve Herman, Yrjö Roos, Magdalena Andryszkiewicz, Ana Gomes, Natalia Kovalkovicova, Yi Liu and Andrew Chesson

Abstract

The food enzyme triacylglycerol lipase (triacylglycerol acylhydrolase; EC 3.1.1.3) is produced with the genetically modified *Aspergillus luchuensis* strain FL105SC by Advanced Enzyme Technologies Ltd. The genetic modifications do not give rise to safety concerns. The food enzyme is free from viable cells of the production organism and its DNA. It is intended to be used in an immobilised form for the production of modified fats and oils by interesterification. Genotoxicity tests did not indicate a safety concern. The systemic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rats. The Panel identified a no observed adverse effect level (NOAEL) of 783 mg TOS/kg body weight per day. As the food enzyme is used in an immobilised form and as any residual amounts of the food enzyme–total organic solids (TOS) are removed during the food manufacturing process, dietary exposure was not calculated. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and no match was found. The Panel considered that a risk of allergic reactions upon dietary exposure cannot be excluded, but the likelihood 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, triacylglycerol lipase, triacylglycerol acylhydrolase, EC 3.1.1.3, lipase, *Aspergillus niger*, genetically modified microorganism

Requestor: European Commission

Question number: EFSA-Q-2015-00561

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.

Suggested citation: EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré, C., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I-L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Herman, L., Roos, Y., … Chesson, A. (2023). Safety evaluation of the food enzyme triacylglycerol lipase from the genetically modified *Aspergillus luchuensis* strain FL105SC. *EFSA Journal*, *21*(10), 1–12. https://doi.org/10.2903/j.efsa.2023.8259

ISSN: 1831-4732

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

Table of contents

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 EU 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 1332/2008 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[3] 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 elements 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, 11.3.2011, pp. 7–15.

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 *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) 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 triacylglycerol lipase from the genetically modified *Aspergillus niger* agg. strain FL105SC.

Recent data identified the production microorganism as *Aspergillus luchuensis* (Section 3.1). Therefore, this name will be used in this opinion instead of *Aspergillus niger* agg.

2. Data and Methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme triacylglycerol lipase from a genetically modified *A. niger* strain FL105SC. The dossier was updated on 24 February 2015.

Additional information was requested from the applicant during the assessment process on 24 April 2019, 9 October 2019 and 12 May 2023, and received on 2 July 2019, 3 January 2020 and 31 May 2023, respectively (see 'Section 5').

Following the request for additional data sent by EFSA on 24 April 2019, EFSA requested a clarification teleconference on 22 June 2023, after which the applicant provided additional data on 6 July 2023.

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 guidance documents of the EFSA Scientific Committee.

The 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA CEF Panel, 2009) as well as the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) 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

IUBMB nomenclature	Triacylglycerol lipase
Systematic name	Triacylglycerol acylhydrolase
Synonyms	Lipase; triglyceride lipase
IUBMB No	EC 3.1.1.3
CAS No	9001-62-1
EINECS No	232-619-9

At low water content, triacylglycerol lipases catalyse the rearrangement of the position of fatty acids in triacylglycerol molecules. The food enzyme under assessment is intended to be used in an immobilised form for the production of modified fats and oils by interesterification.

3.1. Source of the food enzyme

The triacylglycerol lipase is produced with the genetically modified filamentous fungus *Aspergillus luchuensis* strain FL105SC, which is deposited as *Aspergillus niger* agg. at the American Type Culture

Collection (ATCC) with the deposition number [redacted]. The production stain was identified as *A. luchuensis* [redacted].

The culture filtrate from the production strain was examined for the presence of a range of secondary metabolites [redacted]. None were detected.[4]

3.1.1. Characteristics of the parental and recipient microorganisms

The recipient strain is [redacted].

3.1.2. Characteristics of introduced sequences

The sequence encoding the triacylglycerol lipase is [redacted].[5]

3.1.3. Description of the genetic modification process

[redacted].[6] [redacted].[5]

3.1.4. Safety aspects of the genetic modification

The technical dossier contains all necessary information on the recipient microorganism, the donor organism and the genetic modification process.

The production strain *A. luchuensis* FL105SC differs from the recipient strain by its capacity to produce triacylglycerol lipase [redacted].[7]

No issues of concern arising from the genetic modifications 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[8], with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current good manufacturing practice.[9]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, batch fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in

[4] Technical dossier/1st submission/Annex I1.
[5] Technical dossier/1st submission/Annex M.
[6] Additional information July 2019/Annexure A1.
[7] Technical dossier/1st submission/Annex M 1.2.
[8] 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.
[9] Technical dossier/1st submission/p. 31, p. 36/Annex F.

which the enzyme protein is retained, while most of the low molecular mass material passes the filtration membrane and is discarded. Finally, the food enzyme was spray-dried prior to analysis.[10] The applicant provided information on the identity of the substances used to control the fermentation and in the subsequent downstream processing of the food enzyme.[11]

The food enzyme is used in an immobilised form. Immobilisation is achieved by adsorption onto [redacted] resin. The resin beads are then washed and dried.

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

The triacylglycerol lipase is a single polypeptide chain of 291 amino acids.[12] The molecular mass derived from the amino acid sequence was calculated to be 37 kDa.[13] The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.[14] A consistent protein pattern was observed across all batches. The gel showed a single major protein band corresponding to an apparent molecular mass of about 37 kDa, consistent with the expected mass of the enzyme. No other enzyme activities were reported.[15]

The in-house determination of triacylglycerol lipase activity is based on hydrolysis of tributyrin, (reaction conditions: pH 7.0, 30°C, 5 min). The enzyme activity is determined by measuring the release of butyric acid. One lipase unit (LU) is defined as the quantity of enzyme that will produce 1 μmol of butyric acid per minute under the conditions of the assay.[16]

The food enzyme has a temperature optimum around 40°C (pH 7.0) and a pH optimum around pH 10.0 (30°C). Thermostability was tested after a pre-incubation of the food enzyme for 120 min at different temperatures. Under the conditions of the applied temperature stability assay, the activity decreased above 60°C, showing no residual activity above 90°C.[17]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three food enzyme batches used for commercialisation, one of which (batch 3) was used for the toxicological tests (Table 1).[18] The mean total organic solids (TOS) of the three batches was 78.2% and the mean enzyme activity/TOS ratio was 699.1 LU/mg TOS.

Table 1: Compositional data of the food enzyme preparation

Parameter	Unit	Batches		
		1	2	3[(a)]
Triacylglycerol lipase activity	LU/g[(b)]	554,317	536,254	548,458
Protein	%	43.6	38.4	40.7
Ash	%	8.5	9.3	8.8
Water	%	6.7	8.2	7.8
Excipient ([redacted])	%	4.6	6.4	5.2
Total organic solids (TOS)[(c)]	%	80.2	76.1	78.2
Activity/TOS ratio	LU/mg TOS	691.2	704.7	701.4

(a): Batch used for the toxicological studies.
(b): LU: lipase unit (see Section 3.3.1).
(c): TOS calculated as 100% – % water – % ash – % excipient.

[10] Technical dossier/1st submission/pp. 31–37/Annex G_conf.
[11] Technical dossier/1st submission/p. 31, p. 36/Annex G_conf.
[12] Technical dossier/1st submission/pp. 34–35/Annex N.
[13] Technical dossier/1st submission/p. 6/Annex B.
[14] Technical dossier/1st submission/p. 4; Additional information July 2019.
[15] Technical dossier/1st submission/p. 11.
[16] Technical dossier/1st submission/Annex A2.1.
[17] Technical dossier/1st submission/Annex C.
[18] Technical dossier/1st submission/p. 4/Annex A3; Additional information July 2019; Additional information May 2023/Annex_Additional Info_TL Lipase (Strain FL105SC)/Annex A3-TL Lipase_Revised.

3.3.3. Purity

The lead content in all batches was below 0.25 mg/kg,[19] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). In addition, the levels of arsenic, cadmium and mercury were below the limits of quantification (LoQ) of the employed methods.[20]

The food enzyme preparation complies with the microbiological criteria for total coliforms, *Escherichia coli* and *Salmonella*, as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006).[21] No antimicrobial activity was detected in any of these batches.[22]

Strains of *Aspergillus*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Frisvad et al., 2018). The presence of aflatoxins (B1, B2, G1, G2 and M1), ochratoxin A, fumonisin B1, zearalenone, deoxynivalenol, T2-toxin, HT2-toxin, ergocornine, ergocristine, ergocryptine, ergometrine, ergosine and ergotamine was examined in the three food enzyme batches and all were below the limit of detection (LoD) of the applied analytical methods.[23,24] Adverse effects caused by the possible presence of other secondary metabolites were addressed by the toxicological examination of the food enzyme–TOS.

The Panel considered that the information provided on the purity of the food enzyme was sufficient.

3.3.4. Viable cells and DNA of the production strain

The absence of the production strain in the food enzyme was demonstrated [redacted]. No colonies were produced.[25]

The absence of recombinant DNA in the food enzyme was demonstrated [redacted].[26]

3.4. Toxicological data

A battery of toxicological tests, including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian chromosomal aberration test and a repeated dose 90-day oral toxicity study in rats, was provided.

The batch 3 (Table 1) used in these studies was one of the batches used for commercialisation and had similar composition as two other batches; it was considered suitable as a test item.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was performed according to the Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997a) and following good laboratory practice (GLP).[27]

Five strains of Salmonella Typhimurium (TA98, TA100, TA1535, TA102 and TA97a) were used in the presence or absence of metabolic activation (S9-mix), applying the preincubation method. Two experiments were carried out using five concentrations of the food enzyme (from 50 to 5,000 μg food enzyme/plate, corresponding to from 39.15 to 3,915 μg TOS/plate, respectively).

[19] Technical dossier/1st submission/p. 7/Annex D; Additional information July 2019; Additional information May 2023/Annex A3-TL Lipase_Revised.
[20] LoQs: Pb, As, Cd = 0.25 mg/kg each; Hg = 0.025 mg/kg.
[21] Technical dossier/1st submission/p. 7/Annex K1, K2; Additional information May 2023/Annex A3-TL Lipase_Revised.
[22] Technical dossier/1st submission/p. 7/Annex A3; Additional information May 2023/Annex A3-TL Lipase_Revised.
[23] Technical dossier/1st submission/p. 7/Annex E1; Additional information July 2019.
[24] LoDs: aflatoxins (B1, B2, G1, G2 M1) = 1 μg/kg each; ochratoxin A = 1 μg/kg; fumonisin B1 = 0.5 μg/kg; zearalenone 5 μg/kg; deoxynivalenol = 25 μg/kg; T2-toxin = 10 μg/kg; HT2-toxin = 50 μg/kg; ergocornine, erogocristine, ergocryptine, ergometrine, ergosine and ergotamine = 100 μg/kg.
[25] Technical dossier/Annex M 1.3.
[26] Technical dossier/Annex M 1.3/Annex M 1.4; Additional data January 2020.
[27] Technical dossier/1st submission/Annex J.

No evidence of toxicity was observed under any of the conditions tested. Upon treatment with the food enzyme, there was no increase in revertant colony numbers.

The Panel concluded that the food enzyme triacylglycerol lipase did not induce gene mutations under the test conditions employed in this study.

3.4.1.2. *In vitro* mammalian chromosomal aberration test

The *in vitro* mammalian chromosomal aberration test was carried out according to the OECD Test Guideline 473 (OECD, 1997b) and following GLP in cultured human peripheral blood lymphocytes.[27]

Based on the result of a preliminary cytotoxicity assay, three experiments were performed in duplicate cultures. In the first experiment applying 3 h treatment +21 h recovery, the cultures were exposed to concentrations of 500, 1,500 and 5,000 μg food enzyme/mL (corresponding to 392, 1,175 and 3,915 μg TOS/mL) in the absence of metabolic activation (S9-mix). In the second experiment, applying continuous 24 h treatment without S9-mix, the concentrations tested were 100, 300 and 1,000 μg/mL (corresponding to 78.3, 235 and 783 μg TOS/mL). In the third experiment applying 3 h treatment +21 h recovery, the cultures were exposed to concentrations of 500, 1,500 and 5,000 μg/mL (corresponding to 392, 1,175 and 3,915 μg TOS/mL) in the presence of S9-mix.

No significant cytotoxicity was induced by the treatments with the food enzyme. The frequency of structural and numerical aberrations was not statistically significant different to the negative controls at any concentrations tested.

The Panel concluded that the food enzyme triacylglycerol lipase did not induce structural and numerical chromosomal aberrations under the experimental conditions employed for this study.

3.4.2. Repeated dose 90-day oral toxicity study in rodents

A repeated dose 90-day oral toxicity study was performed according to the OECD test guideline 408 (OECD, 1998), and following GLP.[27] Groups of 10 male and 10 female Wistar rats received the food enzyme via gavage at doses of 250, 500 and 1,000 mg/kg body weight (bw) per day, corresponding to 196, 392 and 783 mg TOS/kg bw per day for 90 days. Controls received the vehicle (analytical grade water). Furthermore, a recovery control and a high-dose group were included in the study, each comprising five males and five females and terminated 28 days after the end of the treatment.

No mortality was observed.

In the functional observations, a statistically significantly increased frequency of urination (+67%) and of rearing (+113%) in high-dose males was observed in week 13 of the treatment. The Panel considered the changes as not toxicologically relevant, as they were only observed in one sex and there were no changes in other functional observations' parameters.

Haematological at the end of treatment revealed a statistically significant increase in the total count of white blood cells (WBC) in mid- and high-dose males (+67% and +61%, respectively), an increase in the relative lymphocyte (L) count in mid-dose males (+14%) and a decrease in the platelet count in high-dose females (−30%). The Panel considered the changes as not toxicologically relevant, as they were only observed in one sex (all parameters), there was no dose–response relationship (WBC, L), the change was not present after the recovery period (WBC, L) and the changes were within the historical control values.

Clinical chemistry investigations at the end of the treatment revealed a statistically significant increase in globulin (Glob) concentration in high-dose males (+10%), a decrease in total bilirubin (TB) concentration in mid-dose females (−44%) and a decrease in phosphorous (P) concentration in low- and mid-dose females (−16% and −14%, respectively). The Panel considered the changes as not toxicologically relevant, as they were only observed in one sex (all parameters), there was no dose–response relationship (TB, P) and the changes were within the historical control values (all parameters).

At the end of the recovery period, statistically significant changes were only recorded in the high-dose females: an increase in albumin (+18%) and decreases in concentrations of TB (−11%), sodium (−1%), and calcium (−3%), and in alkaline phosphatase (ALP) activity (−51%). The Panel considered the changes as not toxicologically relevant, as they were only observed in one sex (all parameters), they were not present at the end of the treatment (all parameters) and the changes were within the historical control values (all parameters).

Statistically significant changes in organ weights were observed in the absolute spleen weight (low- and high-dose females, +41% and +65%, respectively), but not in the relative spleen weight. The Panel considered the change as not toxicologically relevant, as it was only observed in one sex, there was no dose–response relationship, there were no histopathological changes in the organ and the change was not present after the recovery period.

No other statistically significant or biologically relevant differences to control were reported.

The Panel identified a no observed adverse effect level (NOAEL) of 783 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity

The allergenicity assessment considered only the food enzyme and not carriers or other excipients that may be used in the final formulation.

The potential allergenicity of the triacylglycerol lipase produced with the genetically modified *A. niger* strain FL105SC was assessed by comparing its amino acid sequence 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 match was found.[28]

No information was available on oral sensitisation or elicitation reactions of this triacylglycerol lipase.

Respiratory allergy following occupational inhalation of triacylglycerol lipase has been reported (Elms et al., 2003; Martel et al., 2010). However, some studies have shown that adults with occupational asthma to an enzyme used in food can commonly ingest the corresponding allergen without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). Adverse reactions upon ingestion of triacylglycerol lipases in individuals sensitised through the respiratory route have not been reported.

[redacted] and [redacted], products that may cause allergies or intolerances (listed in the Regulation (EU) No 1169/2011[29]), are used as raw materials. In addition, [redacted], a known allergen, is also present in the media fed to the microorganisms. However, during the fermentation process, these products will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the fungal biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that no potentially allergenic residues from these sources are present in the food enzyme.

The Panel considered that a risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded, but the likelihood is low.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is used in an immobilised form[30] for the production of modified fats and oils by interesterification.[31]

At low water content, triacylglycerol lipases catalyse the exchange of fatty acids at the sn1- and sn3-position of the triglycerides, modifying the properties of the resulting oils or fats (e.g. melting point and nutritional properties).[32] Interesterified fats can be incorporated into many foods as ingredients, e.g. as alternatives to hydrogenated fats in food formulation, such as spreads, bakery products, biscuits, etc. (Berry et al., 2019).

It is expected that the food enzyme–TOS is absent in the final foods, since the modified fats and oils are further purified by deodorisation and filtration.[33] Furthermore, the applicant provided a laboratory study, which confirmed that no leakage of the immobilised enzyme[34] to the final food was detected, using the total nitrogen content as an approximation of the TOS.[35,36] The technical

[28] Technical dossier/1st submission/Annex L.

[29] 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.

[30] Additional data July 2019/Response 4, Outcome of the clarification June 2023.

[31] Technical dossier/risk assessment data/Section 3.2.1.4A, Section 3.2.1.5; Updated technical dossier May 2023/risk assessment data/Section 3.2.1.5; Additional data July 2019/Response 8.

[32] Technical dossier/risk assessment data/Section 3.2.1.4A.

[33] Technical dossier/risk assessment data/Figure 3.2.1.4-1.

[34] Additional data July 2019/Responses 5 and 6.

[35] Technical dossier/risk assessment data/Annex N.

[36] Additional data July 2019/Response 7. LoD = 1.2 mg nitrogen/g of oil.

information and experimental data on the removal of food enzyme–TOS during the interesterification of fats and oils were considered by the Panel as sufficient to confirm its absence in the final products.

3.5.2. Dietary exposure estimation

The Panel accepted the evidence of TOS removal as sufficient to conclude that the residual food enzyme–TOS in the modified fats and oils is negligible. Therefore, a dietary exposure was not calculated.

3.6. Margin of exposure

Toxicological data were available, but because the dietary exposure was considered negligible, a margin of exposure was not calculated.

4. Conclusions

Based on the data provided, the Panel concluded that the food enzyme triacylglycerol lipase produced with the genetically modified *Aspergillus luchuensis* strain FL105SC does not give rise to safety concerns under the intended conditions of use.

The CEP Panel considered the food enzyme free from viable cells of the production organism and of its recombinant DNA.

5. Documentation provided to EFSA

Application for authorisation of Lipase from a genetically modified *Aspergillus niger* agg. (strain FL105SC). February 2015. Submitted by Advanced Enzyme Technologies Ltd. A consolidated full technical dossier was provided in May 2023.

Additional information. July 2019. Submitted by Advanced Enzyme Technologies Ltd.

Additional information. May 2023. Submitted by Advanced Enzyme Technologies Ltd.

Additional information upon clarification teleconference. July 2023. Submitted by Advanced Enzyme Technologies Ltd.

References

Armentia A, Dias-Perales A, Castrodeza J, Dueñas-Laita A, Palacin A and Fernándes S, 2009. Why can patients with baker's asthma tolerate wheat flour ingestion? Is wheat pollen allergy relevant? Allergologia et Immunopathologia, 37, 203–204.

Berry SE, Bruce JH, Steenson S, Stanner S, Buttriss JL, Spiro A, Gibson PS, Bowler I, Dionisi F, Farrell L, Glass A, Lovegrove JA, Nicholas J, Peacock E, Porter S, Mensink RP and Hall WL. 2019. Interesterified fats: What are they and why are they used? A briefing report from the Roundtable on Interesterified Fats in Foods. Nutrition Bulletin, 44, 363–380.

Brisman J, 2002. Baker's asthma. Occupational and Environmental Medicine, 59, 498–502.

Cullinan P, Cook A, Jones M, Cannon J, Fitzgerald B and Newman Taylor AJ, 1997. Clinical responses to ingested fungal α-amylase and hemicellulase in persons sensitized to *Aspergillus fumigatus*? Allergy, 52, 346–349.

EFSA (European Food Safety Authority), 2009. 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 CEF Panel (EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids), 2009. Guidance of the Scientific Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) on the Submission of a Dossier on Food Enzymes for Safety Evaluation by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids. EFSA Journal 2009;7(8):1305, 26 pp. https://doi.org/10.2903/j.efsa.2009.1305

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), 2019. Statement on the characterisation of microorganisms used for the production of food enzymes. EFSA Journal 2019;17(6):5741, 13 pp. https://doi.org/10.2903/j.efsa.2019.5741

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

Elms J, Fishwick D, Walker J, Rawbone R, Jeffrey P, Griffin P, Gibson M and Curran AD, 2003. Prevalence of sensitisation to cellulase and xylanase in bakery workers. Occupational and Environmental Medicine 60, 802–804.

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

Frisvad JC, Møller LLH, Larsen TO, Kumar R and Arnau J, 2018. Safety of the fungal workhorses of industrial biotechnology: update on the mycotoxin and secondary metabolite potential of *Aspergillus niger*, *Aspergillus oryzae*, and *Trichoderma reesei*. Applied Microbiology and Biotechnology 102, 9481–9515.

Martel C, Nielsen GD, Mari A, Licht TR and Poulsen LK, 2010. Bibliographic review on the potential of microorganisms, microbial products and enzymes to induce respiratory sensitization. EFSA Supporting Publication 2010;7(9):EN-75, 95 pp. https://doi.org/10.2903/sp.efsa.2010.EN-75

OECD (Organisation for Economic Co-Operation and Development), 1997a. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 471: Bacterial reverse mutation test. 11 pp. Available online: http://www.oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en;jsessionid=9zfgzu35paaq.x-oecd-live-01

OECD (Organisation for Economic Co-Operation and Development), 1997b. OECD guideline for the testing of chemicals, Section 4 Health effects, Test No. 473: *In vitro* mammalian chromosomal aberration test. 10 pp. Available online: https://www.oecd-ilibrary.org/environment/test-no-473-in-vitro-mammalian-chromosome-aberration-test_9789264071261-en

OECD (Organisation for Economic Co-Operation and Development), 1998. OECD guideline for the testing of chemicals, Section 4 Health effects, Test No. 408: Repeated dose 90-day oral toxicity study in rodents. 10 pp. Available online: http://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en

Poulsen LK, 2004. Allergy assessment of foods or ingredients derived from biotechnology, gene-modified organisms, or novel food. Molecular Nutrition & Food Research, 48, 413–423.

Abbreviations

ATTC	American Type Culture Collection
bw	body weight
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
DAD	diode-array detector
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
FOA	5-fluoro-orotic acid
GLP	good laboratory practices
GMM	genetically modified microorganism
GMO	genetically modified organism
HPLC	high-performance liquid chromatography
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LOD	limit of detection
LOQ	limit of quantification
LU	lipase unit
MS	mass spectrometry
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
ToF	time of flight
TOS	total organic solids
WHO	World Health Organization