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Safety evaluation of the food enzyme lysophospholipase from *Trichoderma reesei* (strain RF7206)

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Abstract

The food enzyme lysophospholipase (EC 3.1.1.5) is produced with the genetically modified *Trichoderma reesei* strain RF7206 by AB Enzymes GmbH. The genetic modifications do not give rise to safety concerns. The food enzyme is free from viable cells of the production organism and recombinant DNA. The lysophospholipase food enzyme is intended to be used in starch processing for the production of glucose syrups. Residual amounts of total organic solids (TOS) are removed by the purification steps applied during the production of glucose syrups, consequently, dietary exposure was not calculated. Genotoxicity tests did not raise 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 at the highest dose tested of 927 mg TOS/kg body weight (bw) per day. Similarity of the amino acid sequence to those of known allergens was searched and no match was found. The Panel considered that, under the intended condition of use, the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme cannot be excluded, but the likelihood of such reactions to occur is considered to be low. Based on the data provided and the removal of TOS during the intended food production process, the Panel concluded that this food enzyme does not raise safety concerns under the intended conditions of use.

Keywords: food enzyme, lysophospholipase, lecithinase B, EC 3.1.1.5, Lysolecithinase, *Trichoderma reesei*, genetically modified microorganism

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1. Introduction

Article 3 of the Regulation (EC) No 1332/2008[1] provides definitions 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 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:

i) it does not pose a safety concern to the health of the consumer at the level of use proposed;
ii) there is a reasonable technological need;
iii) 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 an approval via an EU Community list.

The 'Guidance on submission of a dossier on a food enzyme for 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 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 companies "Danisco US Inc" for the authorisation of the food enzyme Hexose oxidase from a genetically modified strain of *Hansenula polymorpha* (strain DP-Jza21); "Novozymes A/S" for the authorisation of the food enzyme Pectin lyase from a genetically modified strain of *Aspergillus niger* (strain NZYM-PN); "Puratos NV" for the authorisation of the food enzyme Xylanase from a genetically modified strain of *Bacillus subtilis* (strain LMG-S-27588); the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) for the authorisation of the food enzyme Beta-galactosidase from *Kluyveromyces lactis* and "AB Enzymes GmbH" for the authorisation of the food enzyme Lysophospholipase from a genetically modified strain of *Trichoderma reesei* (strain RF7206).

Following the requirements of Article 12.1 of Commission Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008[2], 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, p. 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, p. 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, p. 15–24.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessment on the food enzymes Hexose oxidase from a genetically modified strain of *Hansenula polymorpha* (strain DP-Jza21), Pectin lyase from a genetically modified strain of *Aspergillus niger* (strain NZYM-PN), Xylanase from a genetically modified strain of *Bacillus subtilis* (strain LMG-S-27588), Beta-galactosidase from *Kluyveromyces lactis* and Lysophospholipase from a genetically modified strain of *Trichoderma reesei* (strain RF7206) in accordance with the 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 food enzyme lysophospholipase from a genetically modified *T. reesei* (strain RF7206).

1.3. Information on existing authorisations and evaluations

The applicant provides non-exhaustive lists of authorised enzymes (other than lysophospholipase) produced by the same production organism – *T. reesei*, and authorised lysophospholipase from production microorganisms other than *T. reesei*.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme lysophospholipase from a genetically modified *T. reesei* (strain RF7206).

Additional information was sought from the applicant during the assessment process in requests from EFSA sent on 19 January 2018 and 16 October 2018 and was consequently provided (see 'Documentation provided to EFSA').

Following the request for additional data sent by EFSA on 19 January 2018, the applicant requested a clarification teleconference, which was held on 21 March 2018.

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) as well as in the EFSA 'Scientific Opinion on Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use' (EFSA GMO Panel, 2011) and following the relevant existing guidance of EFSA Scientific Committee.

The current 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA CEF Panel, 2009) 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:	Lysophospholipase
Systematic name:	2-Lysophosphatidylcholine acylhydrolase
Synonyms:	Lecithinase B
IUBMB No:	EC 3.1.1.5
CAS No:	9001-85-8.

The lysophospholipase catalyses the hydrolysis of an ester bond between a fatty acid and glycerol in lysophospholipids, resulting in the formation of free fatty acids and glycerophosphatide. It is intended to be used in starch processing for the production of glucose syrups.

3.1. Source of the food enzyme[4]

The lysophospholipase is produced with the genetically modified filamentous fungus *T. reesei* strain RF7206 which is deposited in the Centraalbureau voor Schimmelcultures (CBS) with accession number CBS 125079.

3.1.1. Characteristics of the parental and recipient microorganisms

The parental strain is the *T. reesei*

The recipient strain *T. reesei*

The recipient strain was characterised by the CBS as *T. reesei* on the basis of [5]

3.1.2. Characteristics of the introduced sequences

The sequence encoding the

3.1.3. Description of the genetic modification process

The purpose of genetic modification was to enable the production strain to synthesise lysophospholipase from [6]

The recipient strain was modified by [7]

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 *T. reesei* RF7206 differs from the recipient strain only in its capacity to produce the lysophospholipase enzyme from . The presence of the enzyme encoding gene was confirmed by Southern analysis. The phenotypic stability of the *T. reesei* RF7206 strain was confirmed by its capacity to produce a constant level of the enzyme lysophospholipase measured in relation to the TOS in three independent batches of the food enzyme and its genetic stability was demonstrated by Southern analysis of samples taken after multiple (up to 10) generations grown in the absence of selective pressure. [8]

No issues of concern arising from the genetic modifications were identified by the Panel.

[4] Technical dossier/1st submission/Volume III.
[5] Technical dossier/1st submission/Volume III/Appendix 3.
[6] Technical dossier/1st submission/Volume III/Appendix 9.
[7] Technical dossier/2nd submission/Enclosure 3.
[8] Technical dossier/Additional data July 2018/Enclosure 3.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[9], with food safety procedures based on Hazard Analysis and Critical Control Points (HACCP), and in accordance with current Good Manufacturing Practice (GMP).

The production strain is grown as a pure culture using a typical industrial medium in a submerged, fed-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 leaving a supernatant containing the food enzyme. The filtrate containing the enzyme is then further purified and concentrated [redacted] The applicant provided information on the identity and analysis of the substances used to control the fermentation[10,11] and in the subsequent downstream processing of the food enzyme.

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 lysophospholipase is [redacted][12] The molecular mass of the mature protein derived from the amino acid sequence (without the signal peptide) was calculated to be [redacted]. The protein pattern of the food enzyme was investigated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Gels showed a major protein band of about [redacted], which is consistent with the calculated value for the lysophospholipase, and some minor protein bands of lower molecular mass. The food enzyme preparation was tested for xylanase, β-glucanase and cellulase activities, which were determined to be, [redacted][13] No other enzymatic side activities were reported.

The in-house determination of lysophospholipase activity is based on hydrolysis of the substrate lysolecithin (reaction conditions: pH 4.5, temperature 55°C, reaction time 10 min). The enzymatic activity is determined by measuring the release of fatty acids. The lysophospholipase activity is quantified relative to an enzyme standard and expressed in Lysophospholipase Units/g (LPL/g). One unit of lysophospholipase activity corresponds to the amount of enzyme which releases, in a 0.01 M lysolecithin-solution, 1 μmol fatty acids per minute at pH 4.5 and 55°C.[14]

The food enzyme has been characterised with regard to its temperature and pH profiles. It has a temperature optimum around 65°C (pH 5.0) and a pH optimum between pH 4.0 and 5.5 (temperature 55°C). Thermostability was tested after incubation of the food enzyme at different temperatures. Under the conditions (pH 4.35) of the applied temperature stability assay, the lysophospholipase activity decreased rapidly above 65°C showing no residual activity after incubation at 85°C.[15]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme preparation were provided for three batches to be used for commercialisation and one batch used for the toxicological tests (Table 1). The average total organic solids (TOS) of the three food enzyme batches for commercialisation was 7.8% (range 3.7–10.2%). The average enzyme activity/TOS ratio of the three food enzyme batches for commercialisation is 741.

[9] 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, p. 3–21.
[10] Technical dossier/1st submission/Volume II/Annex 11.
[11] Technical dossier/Additional data July 2018.
[12] Technical dossier/1st submission/Volume II/Annex 4.
[13] Technical dossier/2nd submission folder/RF7206 Non suitability Additional data October 2015.
[14] Technical dossier/1st submission/Volume II/Annex 3.
[15] Technical dossier/1st submission/Volume II/Annex 5.

Table 1: Compositional data of the food enzyme preparation.

Parameter	Unit	Batch			
		1	2	3	4[(a)]
Lysophospholipase activity	LPL/g batch[(b)]	35,500	64,800	60,400	666,877
Protein	%	3.0	9.0	7.0	66.2
Ash	%	3.3	5.2	5.3	1.5
Water	%	73.7	50.8	54.1	5.8
Total organic solids (TOS)[(c)]	%	3.7	9.6	10.2	92.7
Activity/mg TOS	LPL/mg TOS	960	675	590	719
Diluents	%	19.3	34.4	30.4	–[(d)]

(a): Batch used for the toxicological studies.
(b): LPL/g: lysophospholipase units/g (see Section 3.1.3).
(c): TOS calculated as 100% – % water – % ash – % diluents.
(d): The batch is a dried enzyme concentrate and does not contain any diluent (containing sodium chloride) or other formulation ingredient.

3.3.3. Purity

The lead content in the three commercial batches and in the batch used for toxicological studies was below 0.05 mg/kg which complies with the specification for lead (< 5 mg/kg) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006).[16]

The food enzyme complies with the microbiological criteria as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006), which stipulate that *Escherichia coli* and *Salmonella* species are absent in 25 g of sample and total coliforms should not exceed 30 colony forming unit (CFU) per gram. No antimicrobial activity was detected in any of these batches (FAO/WHO, 2006).[17]

The presence of mycotoxins (aflatoxins (B1, B2, G1 and G2), sterigmatocystin, ochratoxin A, deoxynivalenol, T2 toxin, HT-2 toxin, fumonisin (B1 and B2) and zearalenone) was examined in the four food enzyme preparation batches. The levels of these mycotoxins were found to be below the limits of detection.[18]

Strains of *Trichoderma*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Blumenthal, 2004) including trichodermin (Watts et al., 1988). The applicant did not provide information on secondary metabolites, other than the mycotoxins indicated above, produced under the conditions of fermentation which might contribute to the food enzyme TOS. This issue is addressed by the toxicological examination of the food enzyme TOS.

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

3.3.4. Viable cells and DNA of the production strain

The absence of the production strain in the food enzyme was demonstrated in three independent batches analysed in triplicate. [redacted][19]

A test for recombinant DNA in the food enzyme was made by polymerase chain reaction (PCR) analysis of three batches in triplicate. No DNA was detected [redacted][20]

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 has

[16] Technical dossier/1st submission/Volume II/Annexes 1 and 2, Additional data July 2018. LOD = 0.05 mg/kg sample solution.
[17] Technical dossier/1st submission/Volume II/Annexes 1 and 2, Additional data July 2018. LODs aflatoxins B1, B2, G1 and G2: 0.05 μg/kg each; sterigmatocystin: 10 μg/kg; ochratoxin A: 2 μg/kg; deoxynivalenol: 70 μg/kg; T2-toxin, HT-2-toxin: 20 μg/kg each; fumonisin B1 and B2: 10 μg/kg each; zearalenone: 50 μg/kg.
[18] Technical dossier/Additional data July 2018/Enclosure 2.
[19] Technical dossier/1st submission/Volume III/Appendices 6 and 20, and Additional data July 2018/Enclosure 4.
[20] Technical dossier/Additional data July 2018/Enclosure 5 and November 2018.

been provided. The batch 4 (Table 1) used in these studies was a dried product with an activity/mg TOS comparable to the three batches for commercialisation. Its use is considered suitable for toxicological testing.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was made according to OECD Test Guideline 471 (OECD, 1997a) and following Good Laboratory Practice (GLP).[21] Five strains of *Salmonella* Typhimurium (TA1535, TA1537, TA98, TA100 and TA102) were used in the presence or absence of metabolic activation, applying the direct plate incorporation method (experiment 1) and the pre-incubation method (experiment 2). The experiments were carried out in triplicate using the following amounts of the food enzyme: 3, 10, 33, 100, 333, 1,000, 2,500 and 5,000 μg/plate, corresponding to 2.7, 9.3, 31, 93, 309, 927, 2,318 and 4,635 μg TOS/plate in the first experiment and 33, 100, 333, 1,000, 2,500 and 5,000 μg/plate, corresponding to 31, 93, 309, 927, 2,318 and 4,635 μg TOS/plate in the second experiment. No significant growth inhibition was observed in any strain at any dose level tested. Upon treatment with the food enzyme, there was no significant increase in the number of revertant colony numbers above the control values in any of the strains with or without S9-mix.

The Panel concluded that the food enzyme 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.[22] Duplicate cultures of Chinese hamster V79 cells were treated with the food enzyme or appropriate positive controls, both in the presence and absence of metabolic activation. Two experiments were performed applying (i) a short-term treatment for 4 h followed by 14 h of recovery in the presence and absence of S9-mix (experiment 1); and (ii) a short-term treatment (4+14 h) with S9-mix and a continuous treatment (18+0 h) without S9-mix (experiment 2). Based on the results of a preliminary cytotoxicity test, the cells were treated with 1,250, 2,500 and 5,000 μg food enzyme/mL (corresponding to 1,159, 2,318 and 4,635 μg TOS/mL) in all the experimental conditions. Two hundred metaphases were scored per experimental point for the analysis of structural chromosomal aberrations. Neither precipitation in culture medium nor significant changes in osmolarity and pH were detected. No significant cytotoxicity was observed up to the highest concentration. In all the tested conditions, the frequency of cells with structural and numerical chromosomal aberrations in treated cultures was comparable to the values detected in the negative controls; exception was the statistically significant increase of aberrations (2%) observed after short treatment in the presence of S9-mix at 5,000 μg/mL (4,635 μg TOS/mL); however, this value was within the historical negative control range (0–4% aberrant cells, excluding gaps) and was not considered biologically relevant.

The Panel concluded that the food enzyme lysophospholipase did not induce structural and numerical chromosome aberrations in cultured Chinese hamster V79 cells, under the test conditions employed for this study.

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

The repeated dose 90-day oral toxicity study was performed in accordance with OECD Test Guideline 408 (OECD, 1998) and following GLP. Groups of 10 male and 10 female Wistar rats received via gavage the food enzyme in doses of 92.7, 278.1 or 927.0 mg TOS/kg body weight (bw) per day. Controls received the vehicle (water).

No mortality was observed.

Kinked tail, transient hair loss and scabs in isolated rats without dose dependence were considered by the Panel as non-treatment related findings.

The mean daily feed intake in all treated male groups from days 15 to 22 onwards (except on days 29–43 in low-dose males) and in all treated female groups from days 36 to 43 onwards tended to be

[21] Technical dossier/1st submission/Volume II/Annex 16.
[22] Technical dossier/1st submission/Volume II/Annex 17.

lower than in the controls. As the differences to controls were small and the lower feed intake was not accompanied by changes in body weight, this finding was considered by the Panel of no toxicological significance.

The strength of the forelimb grip in all treated female groups and of the hind limb grip in mid-dose females were statistically significantly reduced compared to controls. The locomotor activity of high-dose males during the 20–30 min measurement interval was statistically significantly elevated as compared to that of controls. The Panel considered these findings to be of no toxicological relevance as they occurred without dose dependency and were limited to one sex.

Among haematological parameters, the only statistically significant difference to controls was an increased prothrombin time in low-dose females. In view of lacking dose dependency, occurrence in one sex only and that the value was within the range of historical control data from the laboratory, the Panel considered this finding as incidental.

Statistically significant differences to controls in clinical chemistry parameters for males included an increase in sodium (dose-related) and in calcium in all treated groups and increased chloride and cholesterol levels in the high-dose group. For females statistically significant differences included increased calcium in the low-dose group and increased urea, bilirubin and sodium in the high-dose group. As the changes in all the parameters (except for sodium in males) lacked the apparent dose–response, did not correlate with histopathology findings and all values were within the historical control ranges for the laboratory, the Panel considered these findings to be of no toxicological relevance.

Urinalysis revealed statistically significantly elevated urinary bilirubin in high-dose females. As this finding lacked dose–response relationship, consistency between sexes and the value was within the historical control range for the laboratory, the Panel considered this finding as not toxicologically relevant.

Analysis of organ weights revealed a statistically significantly lower absolute heart weight in high-dose males as compared to the controls, which, according to the Panel, could be explained by the (not statistically significant) lower body weights in this group (429 g vs 436 g in the control group).

In treated females, a statistically significantly lower mean absolute kidney weight and kidney-to-brain weight ratio in all treated groups and of kidney-to-body weight ratio in low- and high-dose groups were recorded. Additionally, statistically significantly lower uterus-to-brain ratio in the low-dose group and lower absolute and relative ovaries weights in the mid-dose group were recorded as compared to controls. The Panel considered the changes in organ weights of treated females to be of not toxicological relevance as these were not correlated with any histopathological changes and in case of kidneys with any functional changes (see urinalysis) and they were limited to one sex only.

No other effects were observed.

Overall, the Panel identified a no observed adverse effect level (NOAEL) of 927 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity[23]

The allergenicity assessment considers only the food enzyme and not any carrier or other excipient which may be used in the final formulation.

The potential allergenicity of lysophospholipase produced with the genetically modified *T. reesei* strain RF7206 was assessed by comparison of 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, 2017). Using higher than 35% identity in a sliding window of 80 amino acids as criterion, no match was found.

No information is available on oral sensitisation or elicitation reactions of this lysophospholipase. Several studies have shown that adults with occupational asthma may be able to ingest respiratory allergens without acquiring clinical symptoms of food allergy (Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). In addition, no allergic reactions upon dietary exposure to any lysophospholipase have been reported in the literature. Therefore, it can be concluded that the likelihood of an allergic reaction upon oral ingestion of lysophospholipase produced with the genetically modified *T. reesei* strain RF7206, in individuals respiratory sensitised to lysophospholipase, cannot be excluded, but the likelihood of such a reaction to occur is considered to be low.

[23] Technical dossier: Volume II, Annex 18.

According to the information provided, substances or products that may cause allergies or intolerances (Regulation EU 1169/2011)[24] are used as raw materials in the growth medium of the production organism. However, during the fermentation process, these products will be degraded and utilised by the fungus for cell growth, cell maintenance and production of enzyme protein. In addition, the fungal biomass and fermentation solids will be removed. Considering the fermentation process and downstream processing, the Panel considered that potentially allergenic residues of these foods employed as protein sources are not expected to be present.

Quantifying the risk for allergenicity is not possible in view of the individual susceptibility to food allergens. Allergenicity can be ruled out only if the proteins are fully removed. In the starch processing for the production of glucose syrups, experimental data showed a significant removal (> 99%) of protein. However, traces of protein could be present in glucose syrup.

The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme cannot be excluded but the likelihood of such reactions to occur is considered to be low.

3.5. Dietary exposure

The lysophospholipase is intended to be used in starch processing for the production of glucose syrups at an intended use level of up to 1 mg TOS/kg starch.

In starch processing, the lysophospholipase is added after liquefaction, during the saccharification step, in order to remove the emulsifying properties of the lysophospholipids which are water-soluble and, thus, improve filtration rates.

Experimental data have been provided showing the removal (> 99%) of protein in the course of starch processing for the production of glucose syrups (Documentation provided to EFSA No 4). The Panel considered the evidence as sufficient to conclude that residual amounts of TOS (including substances other than proteins) are removed (> 99%) by the purification steps applied to the production of glucose syrups, i.e. filtration, ion exchange chromatography, treatment with active carbon.

As residual amounts of TOS are removed by the purification steps applied during the production of glucose syrups (by > 99%), a dietary exposure was not calculated.

4. Conclusions

Based on the data provided and on the removal of TOS during the intended food production process, the Panel concluded that the food enzyme lysophospholipase produced with the genetically modified *T. reesei* strain RF7206 does not give rise to safety concerns under the intended conditions of use.

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

Documentation provided to EFSA

1) Dossier "Application for authorisation of a lysophospholipase from a genetically modified strain of *Trichoderma reesei* in accordance with Regulation (EC) No 1331/2008", November 2015. Submitted by AB Enzymes GmbH.
2) Additional information was received from AB Enzymes in July 2018.
3) Additional information was received from AB Enzymes in November 2018.
4) Additional information on "Food enzyme removal during the production of cereal based distilled alcoholic beverages" and "Food enzyme carry/over in glucose syrups". February 2017. Provided by the Association of Manufacturers and Formulators of Enzyme Products.

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Abbreviations

AMFEP	Association of Manufacturers and Formulators of Enzyme Product
bw	body weight
CAS	Chemical Abstracts Service
CBS	Centraalbureau voor Schimmelcultures
CEF	EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
CFU	colony forming units
FAO	Food and Agricultural Organization
GLP	Good Laboratory Practice
GM	genetically modified
GMO	genetically modified organisms
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points

IUBMB	International Union of Biochemistry and Molecular Biology
LPL	Lysophospholipase Units
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
SDS–PAGE	sodium dodecyl sulfate–poly acrylamide gel electrophoresis
TOS	total organic solids
WHO	World Health Organization