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Safety evaluation of the food enzyme α-amylase from the genetically modified *Pseudomonas fluorescens* strain BD15754

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Abstract

The food enzyme α-amylase (4-a-D-glucan glucanohydrolase; EC 3.2.1.1) is produced with the genetically modified *Pseudomonas fluorescens* strain BD15754 by BASF Enzymes LLC1. The food enzyme is free from viable cells of the production organism and recombinant DNA. The α-amylase is intended to be used in distilled alcohol production and starch processing for the production of glucose syrups. Residual amounts of total organic solids (TOS) are removed by distillation and 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 (NOAEL) at the highest dose of 887 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 conditions of use, the risk of allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but the likelihood of such reactions to occur is considered to be low. However, the food enzyme contains residual amounts of a highly important antimicrobial for human medicine, with the consequent risk of promoting the development of resistance. Therefore, the Panel concludes that the food enzyme α-amylase, produced with the genetically modified *P. fluorescens* strain BD15754 cannot be considered safe.

Keywords: food enzyme, α-amylase, 1,4-a-D-glucan glucanohydrolase, EC 3.2.1.1, *Pseudomonas fluorescens*, genetically modified microorganism

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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 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 1331/2008[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 European Union market and intended to remain on that market, as well as all new food enzymes, shall be subjected to a safety evaluation by the European Food Safety Authority (EFSA) and approval via an EU Community list.

The Guidance on submission of a dossier on food enzymes for safety evaluation (EFSA, 2009a) lays down the administrative, technical and toxicological data required.

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background as provided by the European Commission

Only food enzymes included in the European Union (EU) Community list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7 (2) of Regulation (EC) No 1332/2008 on food enzymes.

Five applications have been introduced by the companies "BASF Enzymes LLC1" for the authorisation of the food enzyme Alpha-amylase from a genetically modified strain of *Pseudomonas fluorescens* (BD15754), "DSM Food Specialties B.V." for the authorisation of the food enzyme Phospholipase C from a genetically modified strain *of Pichia pastoris* (PRF), and "Danisco US Inc." for the authorisation of the food enzymes Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb25), Xylose isomerase from a genetically modified strain of *Streptomyces rubiginosus* (DP-Pzn37), and Alpha-amylase from a genetically modified strain of *Bacillus amyloliquefaciens* (DP-Czb53).

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 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, 11.3.2011, p. 15–24.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes Alpha-amylase from a genetically modified strain of *Pseudomonas fluorescens* (strain BD15754), Phospholipase C from a genetically modified strain of *Pichia pastoris* (strain PRF), Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (strain DP-Dzb25), Xylose isomerase from a genetically modified strain of *Streptomyces rubiginosus* (strain DP-Pzn37), and Alpha-amylase from a genetically modified strain of *Bacillus amyloliquefaciens* (strain DP-Czb53) 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 food enzyme α-amylase from a genetically modified *P. fluorescens* (strain BD15754).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme α-amylase from a genetically modified *P. fluorescens* strain BD15754.

Additional information was sought from the applicant during the assessment process on 28 January 2019, and was consequently provided (see 'Documentation provided to EFSA').

Following the reception of additional data by EFSA on 22 November 2019, EFSA requested a clarification teleconference on 20 January 2020, after which the applicant provided additional data on 21 January 2020.

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) 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).

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.

3. Assessment

IUBMB nomenclature:	α-amylase
Systematic name:	4-α-D-glucan glucanohydrolase
Synonyms:	Glycogenase, endoamylase, 1,4-α-D-glucan glucanohydrolase
IUBMB No.:	EC 3.2.1.1
CAS No.:	9000-90-2
EINECS No.:	232-565-6

The α-amylase catalyses the hydrolysis of α−1,4-glycosidic linkages in starch (amylose and amylopectin), glycogen and related polysaccharides and oligosaccharides, resulting in the generation of glucose, soluble dextrins and other oligosaccharides. It is intended to be used in distilled alcohol production and starch processing for glucose syrups production.

3.1. Source of the food enzyme[4]

The α-amylase is produced with the genetically modified bacterium *P. fluorescens* strain BD15754, which is deposited at the American Type Culture Collection (ATCC) with the deposit number [redacted][5] It was identified as *P. fluorescens* [redacted][6] *P. fluorescens* is a Gram-negative bacterium, ubiquitous in nature and particularly in plants and spoiled food, and also colonises mammalian hosts, where it can cause opportunistic infections.

[4] Technical dossier/Annex E.
[5] Technical dossier/Annex E-8.
[6] Technical dossier/Annex E-2.

3.1.1. Characteristics of the parental and recipient microorganisms

The recipient microorganism is *P. fluorescens* Biovar I strain MB101. It is a natural isolate, which has not undergone any modification.

3.1.2. Characteristics of introduced sequences

[redacted][5]

3.1.3. Description of the genetic modification process

[redacted][8]

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.

[redacted]

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[10], with food safety procedures based on Hazard Analysis and Critical Control Points and in accordance with current Good Manufacturing Practice.[11]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, batch or fed-batch fermentation system with conventional process controls in place. [redacted] After completion of the fermentation and release of the intracellular enzyme, the solid biomass is removed from the fermentation broth by filtration leaving a supernatant containing the food

[7] Technical dossier/Annex E-3.
[8] Technical dossier/Annex E-6.
[9] Technical dossier/Annex E-9.
[10] 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.
[11] Technical dossier/Annex F.

enzyme. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in which enzyme protein is retained while most of the low molecular weight material passes the filtration membrane and is discarded. [redacted] 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.[12]

The Panel considered that that the information provided on the manufacturing process and the quality assurance system implemented by the applicant is sufficient to conduct the assessment. The addition of antimicrobials considered by WHO as critically or highly important for human medicine (WHO, 2017), during the manufacturing of the food enzyme, is a safety concern that is further discussed in this opinion.

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The α-amylase is a single polypeptide chain of [redacted] amino acids.[4] The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be 49.7 kDa. The food enzyme was analysed by mass spectrometry analysis. All samples showed a main peak at about 49.6 kDa.[13] No other enzymatic side activities were reported.

The in-house determination of α-amylase activity is based on the ability of the substrate (soluble starch) to complex with iodine, acquiring a blue colour. The enzymatic activity is determined by measuring the decrease in blue colour due to the hydrolysis of the soluble starch (reaction conditions: pH 5.4, 40°C, 30 min). The degree of hydrolysis is determined by comparing the blue value of the hydrolysed starch substrate with that of a colour standard. One Modified Wohlgemuth Unit (MWU) is the activity which hydrolyses one mg of soluble starch to a defined blue value under the conditions of the assay.[14]

The food enzyme has a temperature optimum around 80°C (pH 5.4) and a pH optimum around pH 5.5 (40°C). Thermostability was tested after a pre-incubation of the food enzyme at 105°C for different times. Under the conditions of the applied temperature stability assay, α-amylase activity decreased asymptotically to ca. 40% after ca. 160 min of pre-incubation.

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for four food enzyme batches, three batches of food enzyme after formulation used for commercialisation and one batch produced for the toxicological tests (Table 1). The mean Total Organic Solids (TOS) of the three food enzyme batches for commercialisation was 2.9%. The mean enzyme activity/TOS ratio of the three food enzyme batches for commercialisation is 5,391 MWU/mg TOS.

Table 1: Compositional data of the food enzyme preparation

Parameter	Unit	Batches			
		1	2	3	4[(a)]
α-Amylase activity	MWU/g batch[(b)]	149,977	164,465	153,900	4,230,000
Protein	%	1.4	1.3	1.8	60.0
Ash	%	0.3	0.3	0.4	9.7
Water	%	58.9	59.0	58.6	1.6
Excipient[(c)]	%	38.1	38.0	37.6	0.0
Total Organic Solids (TOS)[(d)]	%	2.7	2.7	3.4	88.7
Activity/mg TOS	MWU/mg TOS	5,555	6,091	4,526	4,769

(a): Batch used for the toxicological studies.
(b): MWU: Modified Wohlgemuth Unit (see Section 3.3.1).
(c): Citric acid, glycerol, methyl paraben, potassium sorbate, trisodium citrate or water.
(d): TOS calculated as 100% – % water – % ash – % Excipient.

[12] Technical dossier/Annex H.
[13] Technical dossier. Section 3.2.1.1.2.1.
[14] Technical dossier/Annex B.

3.3.3. Purity

The lead content in the three commercial batches and in the batch used for toxicological studies was below 0.5 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).[15] In addition, the levels of arsenic, cadmium and mercury were below the limits of detection of the employed methodologies.[16] The food enzyme contains trace amounts of [redacted][17]

The food enzyme preparation 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 *E. coli* and *Salmonella* species are absent in 25 g of sample, and total coliforms should not exceed 30 colony forming unit (CFU) per gram.[18]

Two out of three enzyme batches inhibited the growth of an indicator strain of *Bacillus cereus*.[19] This is probably attributable to the presence of [redacted] in the food enzyme, which was found to be on average [redacted][17] a concentration sufficient to be active against commensal and pathogenic bacteria, with the consequent risk of promoting the development of resistance.

Endotoxin was found in the food enzyme at 97,000 EU/mg.[17] This is further considered in Section 3.4.2.

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 viable cells of the production strain in the food enzyme was demonstrated [redacted][17]

The absence of recombinant DNA in the food enzyme was demonstrated [redacted][17]

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 been provided. Batch 4 (Table 1) used in these studies has similar chemical purity, and thus is 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 made according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997a) and following Good Laboratory Practice (GLP).[20] Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *E. coli* WP2uvrA(pKM101) were used in the presence or absence of metabolic activation, applying the 'treat and plate' assay. Two separate experiments were carried out using six different concentrations of the food enzyme (10.0, 33.3, 100.0, 333, 1,000 and 5,000 μg/plate, corresponding to 8.87, 29.5, 88.7, 295, 887 and 4,435 μg TOS/plate). No cytotoxicity was observed at any concentration level of the test substance. Upon treatment with the food enzyme there was no significant increase in revertant colony numbers above the control values in any strain with or without S9-mix in either experiment.

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

[15] Technical dossier/Annex C-1.
[16] LODs: Pb = As = 0.050 mg/kg; Cd = 0.025 mg/kg; Hg = 0.050 mg/kg.
[17] Technical dossier/Additional information November 2019.
[18] Technical dossier/Annex C-2.
[19] Technical dossier/Annex C-3.
[20] Technical dossier/Annex J.

3.4.1.2. *In vitro* mammalian chromosomal aberration test

The *in vitro* mammalian chromosomal aberration test was carried out according to OECD Test Guideline 473 (OECD, 1997b) and following GLP.[21] The food enzyme was tested for its ability to induce chromosomal aberrations in rat lymphocytes with and without metabolic activation (S9 mix) in two separate experiments at concentrations up to 5,000 μg food enzyme/mL (corresponding to 4,450 μg TOS/mL). In a first experiment, a short-term treatment (4 + 20 h), was performed in the presence and absence of the S9 mix: the concentrations scored for the chromosomal aberration test were 0, 1,250, 2,500, and 5,000 μg food enzyme/mL, corresponding to 0, 1,113, 2,225 and 4,450 μg TOS/mL. In a second experiment, a short-term treatment in the presence of S9 and a long-term treatment (24 + 0) in the absence of S9 were performed. Cytotoxic effects were observed for the short-term treatment in the presence of S9 (71.1% mitotic inhibition at 5,000 μg/mL) and for the long-treatment (up to 100% mitotic inhibition at 5,000 μg/mL, 47.4% at 500 μg/mL and 15.8% at 250 and 125 μg/mL). Therefore, the concentrations scored for chromosomal aberration were 0, 125, 250 and 500 μg food enzyme/mL (corresponding to 0, 140, 223 and 445 μg TOS/mL) for 24 h continuous treatment without S9 and 0, 1,000, 2000, and 4,000 μg food enzyme/mL (corresponding to 0, 890, 1,780 and 3,560 μg TOS/mL) for the 4 h treatment with S9. The enzyme preparation did not induce a significant increase in structural or numerical chromosomal aberrations in cultured human blood lymphocytes, in either of the two independently repeated experiments.

The Panel concluded that the food enzyme did not induce chromosomal aberrations in cultured human blood lymphocytes, under the test conditions employed for this study.

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

The repeated dose 90-day oral toxicity study was performed in accordance with OECD Test Guideline 408 (OECD, 1998) and following GLP.[22] Groups of 21 male and 21 female Fischer 344 rats received by gavage the food enzyme in doses corresponding to 88.7, 266.1 and 887 mg TOS/kg body weight (bw) per day. Controls received the vehicle (water).

Two low-dose male and one high-dose female died on days 59, 70 and 54, respectively. Necropsy and microscopic examination revealed lung inflammation and oedema, which was attributable to aspiration of the test material due to a gavage error.

Occasional noisy respiration was observed in some treated animals, which was considered by the authors to be related to the gavage procedure. Some of the treated rats manifested exudative nasal inflammation.

Feed intake of the treated groups was statistically significantly lower as compared to that of controls: in mid-dose males in week 8, in high-dose males in weeks 6, 8 and 13, and in low-dose females in week 10, in mid-dose females in weeks 8 and 10, and in high-dose females in week 2. The intermittent differences in feed intake were considered by the Panel not to be of toxicological significance since they did not affect body weight or body weight gain in the treated groups.

Haematological investigation revealed a statistically significantly higher mean neutrophil count in mid- and high-dose males, which was also associated to the observed nasal exudative inflammation. Therefore, it was considered by the Panel as a treatment-related effect due to irritation of the nasal cavity upon contact with the test material containing endotoxin; but not one caused by a systemic toxicity of the test item.

Clinical chemistry investigation revealed that the alkaline phosphatase (ALP) value was statistically significantly (14%) increased in high-dose males, whereas alanine transaminase (ALT) and aspartate transaminase (AST) values were decreased in low-dose females. The Panel noted that (i) the increase in ALP value was small and not accompanied by changes in liver weight and morphology, and (ii) the decreases in ALT and AST activities were not dose-related. Therefore, these findings were not considered by the Panel as toxicologically relevant.

Urinalysis revealed a statistically significant decrease in urine volume in low-dose males and an increase in specific gravity in low- and mid-dose males. These changes were not considered by the Panel as treatment related due to the lack of dose-response.

There was a statistically significant decrease in the absolute heart weight in low-dose females and an increase in the absolute and relative liver weight in high-dose females. As these changes lacked a dose–response relationship and histopathological correlations, they were considered by the Panel as incidental.

Histopathology showed very slight to slight chronic active inflammation of the nasal tissues in some animals in all groups. In addition, bronchiolo-alveolar inflammation was observed in some animals of all treatment groups, which varied in severity and extent. In the view of the Panel, those effects may

[21] Technical dossier/Annex K.
[22] Technical dossier/Annex M.

be associated to irritating effects of the enzyme due to the presence of endotoxin. Irritation would most likely occur upon contact of the upper respiratory tract with the enzyme provoked by regurgitation of the test material because the gavage procedure.

No other significant effects were observed.

Overall, the Panel identified a no-observed-adverse-effect level (NOAEL) of 887 mg TOS/kg bw per day, the highest dose tested, on the basis of the absence of systemic effects.

3.4.3. Allergenicity

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 the α-amylase produced with the genetically modified *P. fluorescens* strain BD15754 was assessed by comparing its amino acid sequence with those of known allergens according to the scientific opinion on the assessment of allergenicity of genetically modified 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.[23]

A-amylase from *Aspergillus oryzae* (Brisman and Belin, 1991; Sander et al., 1998; Quirce et al., 2002; Brisman, 2002) is known as an occupational respiratory allergen associated with baker's asthma. However, several studies have shown that adults with occupational asthma to a food enzyme can ingest respiratory allergens without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Poulsen, 2004; Armentia et al., 2009). Considering the wide use of α-amylase as a food enzyme, only a low number of case reports have been described in the literature focusing on allergic reactions upon oral exposure to α-amylase in individuals respiratory sensitised to this enzyme (Losada et al., 1992; Quirce et al., 1992; Baur and Czuppon, 1995; Kanny and Moneret-Vautrin, 1995; Moreno-Ancillo et al., 2004). The Panel noted that an allergic reaction upon oral ingestion of the α-amylase produced with the genetically modified *P. fluorescens* strain BD15754, in individuals respiratory sensitised to α-amylase, cannot be ruled out, but the likelihood of such a reaction to occur is considered to be low.

According to the information provided, substances or products that may cause allergies or intolerances (Regulation (EU) No 1169/2011[24]) are used as raw materials ([redacted]) 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 microbial biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that potentially allergenic residues of these food employed as a carbon source is 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 occurring is considered to be low.

3.5. Dietary exposure

The food enzyme is intended to be used in distilled alcohol and starch processing for the production of glucose syrups at recommended use levels of up to 81.3 mg TOS/kg grain or starch.

In distilled alcohol production, the food enzyme is added during the slurry mixing step, in the liquefaction step and if needed in the pre-saccharification step. α-amylase is intended to be used to convert liquefied starch into a maltose-rich solution, to increase the amounts of fermentable sugars which results in higher alcohol yields.

In starch processing, the food enzyme is added to the liquefied starch during the saccharification step in order to reduce viscosity of gelatinised starch and to produce glucose syrups.

[23] Technical dossier/Annex N.

[24] 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.

Experimental data have been provided showing the removal (> 99%) of protein in the course of distilled alcohol production and starch processing for the production of glucose syrups (Documentation provided to EFSA No°5). The Panel considered the evidence as sufficient to conclude that residual amounts of TOS (including substances other than proteins) are removed by distillation. The food enzyme could also not be found in glucose syrups produced using it by enzyme-linked immunosorbent assay (ELISA) (Landry et al., 2003).[25] Taking into account the purification steps applied to the production of glucose syrups, i.e. filtration, ion exchange chromatography, treatment with active carbon, the Panel considers that the amount of TOS in the final glucose syrup will be removed to a similar degree.

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

4. Conclusions

The food enzyme contains residual amounts of [redacted] (a highly important antimicrobial for human medicine), [redacted], with the consequent risk of promoting the development of resistance. Therefore, the Panel concludes that the food enzyme α-amylase produced with the genetically modified *P. fluorescens strain BD15754* cannot be considered safe.

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

Documentation provided to EFSA

1) Application for authorisation of α-amylase from the genetically modified strain *Pseudomonas fluorescens* BD15754. March 2016. Submitted by BASF Enzymes LLC.
2) Additional information, November 2019. Submitted by BASF Enzymes LLC.
3) Additional information, January 2020. Submitted by BASF Enzymes LLC.
4) Summary report on GMM part for alpha-amylase produced by *Pseudomonas fluorescens* strain BD15754, EFSA-Q-2016-00200. March 2018. Delivered by the Technical University of Denmark (Lyngby, Denmark).
5) 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

ALP	Alkaline phosphatase
ALT	Alanine transaminase
AST	Aspartate transaminase
ATCC	American Type Culture Collection
bw	body weight
CAS	Chemical Abstracts Service
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
CFU	colony forming units
EC	Enzyme Commission
EINECS	European Inventory of Existing Commercial Chemical Substances
ELISA	enzyme-linked immunosorbent assay
FAO	Food and Agricultural Organization of the United Nations
GMM	genetically modified microorganism
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
IPTG	isopropyl-β-D-thiogalactopyranoside
JECFA	Joint FAO/WHO Expert Committee on Food Addictives
LOD	limit of detection
MWU	Modified Wohlgemuth Unit
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
TOS	Total Organic Solids
WHO	World Health Organization