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Safety evaluation of the food enzyme α-amylase and 1,4-α-glucan 6-α-glucosyltransferase from *Paenibacillus alginolyticus*

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Abstract

The food enzyme α-amylase (4-α-D-glucan glucanohydrolase, EC 3.2.1.1) and 1,4-α-glucan 6-α-glucosyltransferase ((1→4)-α-D-glucan:(1→4)-α-D-glucan(D-glucose) 6-α-D-glucosyltransferase, EC 2.4.1.24) is produced with a *Paenibacillus alginolyticus* by Hayashibara Co., Ltd. The food enzyme is free from viable cells of the production organism. The α-amylase and 1,4-α-glucan 6-α-glucosyltransferase is intended to be used in starch processing for the production of isomaltodextrins. Residual amounts of total organic solids are removed by the purification steps applied during the production of isomaltodextrins and consequently dietary exposure is considered negligible. Similarity of the amino acid sequences to those of known allergens was searched and no matches were found. The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions by dietary exposure to the food enzyme cannot be excluded, but the likelihood is considered to be 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, α-amylase, EC 3.2.1.1, 1,4-α-glucan 6-α-glucosyltransferase, (1→4)-α-D-glucan:(1→4)-α-D-glucan(D-glucose) 6-α-D-glucosyltransferase, EC 2.4.1.24, *Paenibacillus alginolyticus*

Requestor: European Commission

Question numbers: EFSA-Q-2016-00521 and EFSA-Q-2016-00522

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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 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 companies "Hayashibara Co., Ltd." for the authorisation of the food enzymes Alpha-amylase from *Bacillus circulans*/*Paenibacillus alginolyticus*, 1-4-α-glucan 6-α-glucosyltransferase from *Bacillus circulans*/*Paenibacillus alginolyticus*, Cyclomaltodextrin glucanotransferase from *Bacillus circulans*, Isoamylase from *Pseudomonas arnyloderamosa* and by "Intertek Scientific & Regulatory Consultancy" for the authorisation of the food enzyme D-Fructose 4-epimerase from a genetically modified strain of *Corynebacterium glutamicum* (strain FIS003).

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, 11.03.2011, pp. 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 *Bacillus circulans*/*Paenibacillus alginolyticus*, 1,4-α-glucan 6-α-glucosyltransferase from *Bacillus circulans*/*Paenibacillus alginolyticus*, Cyclomaltodextrin glucanotransferase from *Bacillus circulans*, Isoamylase from *Pseudomonas amyloderamosa*, D-Fructose 4-epimerase from a genetically modified strain of *Corynebacterium glutamicum* (strain FIS003) 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 enzymes α-amylase and 1,4-α-glucan 6-α-glucosyltransferase from *P. alginolyticus*.

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 and 1,4-α-glucan 6-α-glucosyltransferase from *P. alginolyticus*.

Additional information was requested from the applicant during the assessment process on 15 November 2017 and on 4 December 2018 and was consequently provided (see 'Documentation provided to EFSA').

Following the request made by the applicant, a clarification teleconference was held on 29 June 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) and following the relevant existing guidances from the 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

The food enzyme contains two declared activities:

IUBMB nomenclature:	α-amylase
Systematic name:	4-α-D-glucan glucanohydrolase
Synonyms:	Glycogenase; Endoamylase; Takaamylase A; 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 1,4-α-D-glycosidic linkages in starch and related polysaccharides containing three or more (1→4)-α-linked D-glucose units, resulting in the generation of hydrolysed starch, dextrins, oligosaccharides and glucose.

IUBMB nomenclature:	1,4-α-glucan 6-α-glucosyltransferase
Systematic name:	(1→4)-α-D-glucan:(1→4)-α-D-glucan(D-glucose) 6-α-D-glucosyltransferase
Synonyms:	Glycogen g-α-D-glucanohydrolase; 1,4-α-D-glucan 6-α-D-glucosyltransferase; D-glucosyltransferase; Oligoglucan-branching glycosyltransferase; T-enzyme; Transglucosidase; α-Glucosyltransferase; 6-Glucosyltransferase, Branching glycosyltransferase
IUBMB No:	EC 2.4.1.24
CAS No:	9030-12-0
EINECS No:	not available

The 1,4-α-glucan 6-α-glucosyltransferase catalyses the transfer of an α-D-glucosyl residue in a 1,4-α-D-glucan to the primary hydroxyl group of free glucose or glucose residues in 1,4-α-D-glucans.

The food enzyme, consisting of α-amylase and 1,4-α-glucan 6-α-glucosyltransferase activities, is intended to be used in starch processing for the production of isomaltodextrins.

3.1. Source of the food enzyme

The food enzyme is produced with a non-genetically modified (non-GM) *P. alginolyticus*, which is deposited at International Patent Organism Depositary, National Institute of Advanced Industrial Science and Technology, Japan with deposit number FERM BP-10771.[4]

Paenibacillus alginolyticus was originally isolated from soil (Nakamura, 1987). Its identification was based on 16S rRNA analysis (1,477 bp) (Shida et al., 1997). The production strain was derived from the original isolate through conventional mutagenesis.[5]

Some *Paenibacillus* species are known to infect various organisms, including honeybees and have very occasionally been found as opportunistic infections in compromised humans. In common with many species of *Bacillus,* with which they were previously classified, they are also reported to form non-ribosomally produced peptides with surfactant-like activities (Grady et al., 2016). For this reason, the applicant made a cytotoxicity assay [redacted][4]

The production strain was tested for its susceptibility to a battery of antibiotics based on those recommended for the testing of *Bacillus* spp. The minimum inhibitory concentration (MIC) values obtained were in all cases below the cut-off values given in the latest guidance on the testing for antibiotic susceptibility (EFSA FEEDAP Panel, 2018).[4]

No mycotoxins or antibacterial activity was detected in the food enzyme.[6]

3.2. Production of the food enzyme

The food enzyme is manufactured according the Food Sanitation Act in Japan.[7] In addition, the Japan Quality Assurance Organization certifies that the plant and its production process follow a Quality Management System, which complies with the requirements of ISO 9001:2008 and is in accordance with current Good Manufacturing Practice.

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, 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. 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.

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 α-amylase is a single polypeptide chain of [redacted] amino acids and the 1,4-α-glucan 6-α-glucosyltransferase a single polypeptide chain of [redacted] amino acids. The food enzyme was analysed by sodium dodecyl sulfate–poly acrylamide gel electrophoresis (SDS–PAGE) analysis. A consistent protein pattern was observed across all batches. The gels showed two major protein bands with the apparent molecular masses of about 58 and 90 kDa, corresponding to the expected molecular masses of the α-amylase and glucosyltransferase, respectively.[4] Gels were essentially free from other stained bands

[4] Technical dossier/Additional information May 2018.
[5] Technical dossier/Additional information October 2018/Amended technical dossiers.
[6] Technical dossier/Annex 1 and 2 (2016-00521) and Annex 3 and 4 (2016-00522).
[7] Act No. 233 (1947) Food Sanitation Act, Japan. http://law.egov.go.jp/htmldata/S22/S22HO233.html (English translation: http://www.japaneselawtranslation.go.jp/law/detail/?printID=&ft=1&re=01&dn=1&co=01&ia=03&ky=%E9%A3%9F%E5%93%81%E8%A1%9B%E7%94%9F%E6%B3%95&page=6&vm=02).

and separate tests for protease and lipase activities were negative.[8] No other enzymatic side activities were reported.

The in-house determination of α-amylase activity is based on hydrolysis of the substrate dextrin (reaction conditions: pH 6.0, 40°C, and 30 min). After acidification to stop the reaction and the addition of an iodine solution (15 min, 25°C) the absorbance is measured spectrophotometrically at 660 nm. One α-amylase unit (AU) is defined as the quantity of enzyme that causes the iodine-starch reaction of 40 mg of dextrin to completely disappear under the conditions of the assay (at pH 6.0 and 40°C).[9]

The in-house determination of 1,4-α-glucan 6-α-glucosyltransferase activity is based on hydrolysis of the substrate maltose (reaction conditions: pH 6.0, 40°C, 30 min). The enzymatic activity is determined by measuring the release of glucose spectrophotometrically. The enzyme activity is expressed in GTU/mL. One 1,4-α-glucan 6-α-glucosyltransferase (GTU) is defined as the quantity of enzyme that causes the release of 1 μmol glucose from the substrate maltose for one minute under the conditions of the assay.[10]

The α-amylase and 1,4-α-glucan 6-α-glucosyltransferase have been characterised with regard to its temperature and pH profiles. The α-amylase has a temperature optimum of about 55°C (pH 6.0) and a pH optimum of pH 6.0 (35°C). The 1,4-α-glucan 6-α-glucosyltransferase activity shows a similar temperature optimum of about 50°C (pH 6.0) and pH optimum between 5.5 and 6.5 (40°C). When pre-incubated for 60 min, α-amylase activity was retained up to temperatures of 40°C in the absence of calcium and 50°C in the presence of added calcium. No residual activity was found at 60°C regardless of the presence or absence of added calcium. Under the same test conditions, glucosyltransferase activity was also retained at temperatures up to 40°C and lost at 60°C.[11]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for four food enzyme batches, three batches used for commercialisation and one batch produced for the toxicological tests (Table 1).

The three commercial food enzyme batches (batch 1–3) presented in Table 1 are liquid concentrates [redacted]. The fourth batch used in the toxicological tests was free from excipients [redacted].[4] The average total organic solids (TOS) of the three food enzyme batches for commercialisation was 1.5% (range 1.2–1.7%). The average enzyme activity/TOS ratios of the three food enzyme batches for commercialisation are 9.1 α-amylase units (AU)/mg TOS and 92.8 1,4-α-glucan 6-α-glucosyltransferase units (GTU)/mg TOS (Table 1).

Table 1: Compositional data of the food enzyme preparation

Parameter	Unit	Batches			
		1	2	3	4[(a)]
α-Amylase activity	AU/mL batch[(b)]	136	91	198	80
1,4-α-Glucan 6-α-glucosyltransferase activity	GTU/mL batch[(c)]	1,530	574	2,390	1,023
Protein	%	1.07	0.623	1.34	NA[(e)]
Ash	%	12.6	12.7	12.1	0.7
Water	%	85.7	86.1	86.2	96.9
Total organic solids (TOS)[(d)]	%	1.7	1.2	1.7	2.4
α-Amylase activity/mg TOS	AU/mg TOS	8.0	7.6	11.7	3.3
1,4-α-Glucan 6-α-glucosyltransferase activity/mg TOS	GTU/mg TOS	90.0	47.8	140.6	42.1

(a): Batch used for the toxicological studies.
(b): AU: α-amylase unit (see Section 3.3.1).
(c): GTU: 1,4-α-glucan 6-α-glucosyltransferase unit (see Section 3.3.1).
(d): TOS calculated as 100% – % water – % ash.
(e): NA: not analysed.

[8] Technical dossier/Annex 5 (2016-00521)/Annex 7 (2016-00522) and Additional information May 2018.
[9] Technical dossier/Annex 4 (2016-00521).
[10] Technical dossier/Annex 6 (2016-00522).
[11] Technical dossiers/Annex 13.

3.3.3. Purity

The lead content in the three commercial batches and in the batch used for toxicological studies 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).[12]

The food enzyme preparation generally 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 *Salmonella* species are absent in 25 g of sample and that the count of total coliforms should not exceed 30 CFU (Colony Forming Units) per gram. Although *Escherichia coli* was not detected, the method used only allows a conclusion of absence in 3 g test material and not the 25 g specified by FAO/WHO (2006).

No antimicrobial activity was detected in any of these batches (FAO/WHO, 2006).

The presence of aflatoxins B1, B2, G1 and G2 was examined in the three commercial batches of the food enzyme preparation and were below the limits of detection (LoD) of the applied analytical methods.[13]

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

3.3.4. Viable cells of the production strain

The absence of the production strain in the food enzyme was demonstrated [redacted][14]

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 have been provided. The food enzyme batch (batch 4 in Table 1) used for toxicological testing has a higher TOS content and a lower specific activity than the batches used for commercialisation and is considered a suitable test substance. However, the applicant derived the doses from the food enzyme rather than the TOS content as recommended in the explanatory note for the guidance on the submission of a dossier on food enzymes (EFSA CEF Panel, 2014) with the result that the highest dose tested was too low to allow a conclusion on toxicity.

3.4.1. Genotoxicity

3.4.1.1. Bacterial Reverse Mutation test

A bacterial reverse mutation assay (Ames test) was performed according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997a) and following Good Laboratory Practice (GLP).[15] Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *E. coli* WP2uvrA were used, applying the pre-incubation method both in the presence and absence of metabolic activation (S9-mix). Two experiments were performed in triplicate using six different concentrations of the food enzyme (156, 313, 625, 1,250, 2,500 and 5,000 μg/plate, corresponding to 3.7, 7.5, 15.0, 30.0, 60.0 and 120.0 μg TOS/plate). No precipitation and growth inhibition were observed in any strain at any dose level tested. Upon treatment with the food enzyme, there was no significant increase in revertant colony numbers and values were within the historical vehicle control ranges.

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

[12] LoD: 5 mg/kg/Technical dossier/Additional Information May 2018.
[13] LoD: 1.0 μg/kg/Technical dossier/Annex 1 (2016-00521)/Annex 3 (2016-00522).
[14] Technical dossier/Additional information October 2018.
[15] Technical dossier/Annex 23 (2016-00521)/Annex 22 (2016-00522).

3.4.1.2. *In vitro* chromosomal aberration test

The *in vitro* chromosome aberration test was carried out in Chinese hamster lung fibroblast (CHL/IU) cells according to OECD Test Guideline 473 (OECD, 1997b) and following GLP.[16]

The dose-finding study was performed at concentrations ranging from 39.1 to 5,000 μg/mL, and no inhibition of cell growth by 50% or more was observed. Based on these results, the cells were exposed to the food enzyme at 1,250, 2,500 and 5,000 μg/mL (corresponding to 30.0, 60.0 and 120.0 μg TOS/mL, in a short-term treatment (6 h followed by 18 h recovery period) with and without metabolic activation (S9-mix), and a continuous treatment (24 h) in the absence of S9-mix. The relative cell growth rate at 5,000 μg/mL was 125, 94 and 113% of negative control values in the short-term treatment −S9, +S9 and continuous treatment, respectively. The frequency of chromosomal aberrations in treated cultures was comparable to the values detected in negative controls and within the range of the laboratory historical solvent control data.

The Panel concluded that food enzyme did not induce chromosome aberrations under the test conditions employed for this study.

3.4.2. Repeated Dose 90-day Oral Toxicity Study

A repeated dose 90-day oral toxicity study was performed according to OECD Test Guidelines 408 (OECD, 1998) and following GLP.[17] Groups of 10 male and 10 female Crl:CD(SD) rats received daily by gavage the food enzyme at doses of 100, 300 and 1,000 mg/kg per day, corresponding to 2.4, 7.2 and 24.0 mg TOS/kg body weight (bw) per day. A control group received the vehicle (purified water) alone. The doses were set on the basis of results of a 14 day dose-range finding study in rats in which no adverse effects were seen with the top dose tested (24 mg TOS/kg bw per day).

One low-dose male died unexpectedly on day 80. The cause of death was not established.

Locomotor activity of males and females after administration of the test substance appeared to decrease with increasing dose; the difference to controls was statistically significant at 40–50 min and 10–20 min, in high-dose males and females, respectively. In high-dose females, also total amount of movement (0–60 min after administration) was statistically significantly lower than that in the control group. In the absence of any other signs indicating the influence of the treatment on the nervous system, the changes in locomotor activity were considered not to be toxicologically significant.

Statistically significant differences from controls in haematological parameters were recorded for females only and included increased values of mean corpuscular haemoglobin and mean corpuscular volume at the mid-dose, and higher percent of basophiles in the high-dose group. As the changes lacked dose response and the increase in basophils was minimal these changes were considered by the Panel not to be toxicologically significant.

Among blood chemistry parameters in high-dose females, β-globulin ratio was statistically significantly higher than in the control group, while the total bilirubin, total protein and albumin concentrations were statistically significantly lower than in the control group. These changes were considered by the Panel not to be toxicological significant because the differences from controls were small and the values were within the historical control data from the laboratory.

In low-dose males, the absolute and relative weights of the thyroid glands were statistically significantly lower than those in controls. However, the absolute and relative weights of the thymus were higher than those in the control group. These not dose-related changes were considered by the Panel as incidental.

No other treatment-related effects were observed.

The Panel identified a no observed adverse effect level (NOAEL) of 24.0 mg TOS/kg bw per day, the highest dose tested.

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.

Potential allergenicity of α-amylase and 1,4-α-glucan 6-α-glucosyltransferase produced with *P. alginolyticus* has been assessed by comparing their amino acid sequences with those of known allergens according to the scientific opinion on the assessment of allergenicity of GM plants and

[16] Technical dossier/Annex 24 (2016-00521)/Annex 23 (2016-00522).
[17] Technical dossier/Annex 25 (2016-00521)/Annex 24 (2016-00522).

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 window of 80 amino acids as the criterion, no match was found.[18]

No information is available on oral and respiratory sensitisation or elicitation reactions of the α-amylase or 1,4-α-glucan 6-α-glucosyltransferase from *P. alginolyticus.*

Several studies have shown that adults with occupational asthma to a food enzyme (as described for α-amylase from *Aspergillus oryzae*) may be able to ingest the corresponding enzyme without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Poulsen, 2004; Armentia et al., 2009). Taking into account the wide use of α-amylase as a food enzyme only a low number of case reports of allergic reactions upon oral exposure to α-amylase in individuals respiratory sensitised to α-amylase (Losada et al., 1992; Quirce et al., 1992; Baur and Czuppon, 1995; Kanny and Moneret-Vautrin, 1995; Moreno-Ancillo et al., 2004) have been described in literature. Therefore, it can be concluded that an allergic reaction upon oral ingestion of α-amylase or 1,4-α-glucan 6-α-glucosyltransferase from *P. alginolyticus* in individuals sensitised to α-amylase or other food enzymes by inhalation cannot be excluded, 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 1169/2011)[19] are used as raw materials ([redacted]) in the media in the production of the food enzyme. However, the [redacted] will be digested during the fermentation process and consumed by the microorganisms for cell growth, cell maintenance and production of enzyme protein. Therefore, potentially allergenic residues of [redacted] are not expected to be present.

The Panel considers 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.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in starch processing to produce isomaltodextrins at a recommended use level of up to 150 mg TOS/kg starch. The applicant confirmed that isomaltodextrins are the only products that are produced by the use of this food enzyme.[20] The food enzyme preparation is typically added during the saccharification step where it degrades gelatinised starch into isomaltodextrins.

Isomaltodextrins are highly branched dextrins, resistant to digestion and thus used as soluble dietary fibres in various processed foods (Watanabe et al., 2018).

Experimental data have been provided on the removal (> 99%) of protein in the course of starch processing for the production of glucose syrup (Documentation provided to EFSA No. 3). Since the purification processes applied to the production of glucose syrup from starch are essentially the same as those used in isomaltodextrin production, the food enzyme would be expected to be similarly removed. The applicant provided evidence confirming the removal of the food enzyme under assessment by an enzyme-linked immunosorbent assay test, [redacted][20]

The Panel considers the evidence as sufficient to conclude that residual amounts of TOS (including substances other than proteins) are removed by the purification steps applied to the production of isomaltodextrins, i.e. filtration, ion exchange chromatography and treatment with active carbon.

3.5.2. Dietary exposure estimation

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

[18] Technical dossier/Annex 9.

[19] 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.

[20] Technical dossier/Additional information January 2019.

4. Conclusions

Based on the data provided and the removal of TOS during the intended food production process, the Panel concluded that the food enzyme α-amylase and 1,4-α-glucan 6-α-glucosyltransferase produced with the *P. alginolyticus* does not give rise to safety concerns under the intended conditions of use.

Documentation provided to EFSA

1) Technical dossier "Application for authorisation of α-amylase from *B. circulans*/*P. alginolyticus* (microorganisms's species-specific authorisation) in accordance with Regulation (EC) No 1331/2008". June 2016. Submitted by Hayashibara Co., Ltd.
2) Technical dossier "Application for authorisation of 1,4-α-glucan 6-α-glucosyltransferase from *B. circulans*/*P. alginolyticus* (microorganisms's species-specific authorisation) in accordance with Regulation (EC) No 1331/2008". June 2016. Submitted by Hayashibara Co., Ltd.
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Abbreviations

AU	α-amylase unit
BLAST	basic local alignment search tool
bw	body weight
CAS	Chemical Abstracts Service
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CFU	colony forming units
CHL/IU	chinese hamster lung fibroblast cells
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
GLP	Good Laboratory Practice
GM	genetically modified
GMO	genetically modified organisms
GTU	1,4-α-glucan 6-α-glucosyltransferase unit
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LoD	limit of detection
MIC	minimum inhibitory concentration
NA	not analysed

NaCl	sodium chloride
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