SCIENTIFIC OPINION

efsa JOURNAL

ADOPTED: 25 January 2023

doi: 10.2903/j.efsa.2023.7833

Safety evaluation of the food enzyme β-fructofuranosidase from the non-genetically modified *Saccharomyces cerevisiae* strain INV

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), Claude Lambré, José Manuel Barat Baviera, Claudia Bolognesi, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis, Holger Zorn, Lieve Herman, Magdalena Andryszkiewicz, Ana Gomes, Yi Liu, Giulio di Piazza and Andrew Chesson

Abstract

The food enzyme β-fructofuranosidase (β-D-fructofuranoside fructohydrolase; EC 3.2.1.26) is produced with the non-genetically modified *Saccharomyces cerevisiae* strain INV by DSM Food Specialties B.V. It is intended to be used in four food manufacturing processes: manufacture of specialty carbohydrates (invert sugar syrups), baking processes, production of sucrose-based fermented beverages and confectionary processes. The dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 2.51 mg TOS/kg body weight (bw) per day in European populations. Toxicological studies were not considered necessary, given the qualified presumption of safety status of the production strain and the nature of the manufacturing process. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and one match with a tomato allergen was found. The Panel considered that, under the intended conditions of use, the risk of allergic reactions upon dietary exposure to this food enzyme, particularly in individuals sensitised to tomato, cannot be excluded. However, the likelihood of allergic reactions to the β-fructofuranosidase from *S. cerevisiae* strain INV is expected not to exceed the likelihood of allergic reactions to tomato. As the prevalence of allergic reactions to tomato is low, also the likelihood of such reaction to occur to the food enzyme is low. Based on the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns, under the intended conditions of use.

Keywords: food enzyme, β-fructofuranosidase, β-D-fructofuranoside fructohydrolase, EC 3.2.1.26, invertase, *S. cerevisiae*

Requestor: European Commission

Question number: EFSA-Q-2021-00695

Correspondence: fip@efsa.europa.eu

Panel members: José Manuel Barat Baviera, Claudia Bolognesi, Andrew Chesson, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Claude Lambré, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis and Holger Zorn.

Note: The full opinion will be published in accordance with Article 12 of Regulation (EC) No 1331/2008 once the decision on confidentiality will be received from the European Commission.

Declarations of interest: If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

Suggested citation: EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré C, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mengelers M, Mortensen A, Rivière G, Steffensen IL, Tlustos C, Van Loveren H, Vernis L, Zorn H, Herman L, Andryszkiewicz M, Gomes A, Liu Y, di Piazza G and Chesson A, 2023. Scientific Opinion on the safety evaluation of the food enzyme β-fructofuranosidase from the non-genetically modified *Saccharomyces cerevisiae* strain INV. EFSA Journal 2023;21(2):7833, 14 pp. https://doi.org/10.2903/j.efsa.2023.7833

ISSN: 1831-4732

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

Table of contents

Abstract 1
1. Introduction 4
1.1. Background and Terms of Reference as provided by the requestor 4
1.1.1. Background as provided by the European Commission 4
1.1.2. Terms of Reference 5
1.2. Interpretation of the Terms of Reference 5
2. Data and methodologies 5
2.1. Data 5
2.2. Methodologies 5
3. Assessment 5
3.1. Source of the food enzyme 6
3.2. Production of the food enzyme 6
3.3. Characteristics of the food enzyme 6
3.3.1. Properties of the food enzyme 6
3.3.2. Chemical parameters 7
3.3.3. Purity 7
3.3.4. Absence of viable cells 7
3.4. Toxicological data 7
3.4.1. Allergenicity 8
3.5. Dietary exposure 8
3.5.1. Intended use of the food enzyme 8
3.5.2. Dietary exposure estimation 9
3.5.3. Uncertainty analysis 10
3.6. Margin of exposure 10
4. Conclusion 10
5. Documentation as provided to EFSA 11
References 11
Abbreviations 12
Appendix A – Dietary exposure estimates to the food enzyme–TOS in details 13
Appendix B – Population groups considered for the exposure assessment 14

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:

- it does not pose a safety concern to the health of the consumer at the level of use proposed;
- there is a reasonable technological need;
- its use does not mislead the consumer.

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

The 'Guidance on submission of a dossier on food enzymes for safety evaluation' (EFSA, 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.

Invertase is an enzyme authorized as a food additive (E 1103) in the context of Regulation (EC) No 1333/2008 on food additives and its specifications are laid down in Commission Regulation (EU) No 231/2012[3] laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008. According to Regulation (EU) No 257/2010[4] setting up a programme for the re-evaluation of approved food additives, invertase shall be re-evaluated by 31 December 2016. However, taking into account that invertase will be transferred from the list of approved additives to the future Union List of approved food enzymes (Article 34 of Regulation (EC) No 1333/2008 and Article 18 of Regulation (EC) No 1332/2008 on food enzymes), the Commission decided that it is more appropriate to evaluate invertase as food enzyme in accordance with Regulation (EC) No 1332/2008. The food enzyme will be evaluated once a detailed schedule has been prepared to include specific deadlines for EFSA's safety evaluations and all the applications have been validated by the Commission.

[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 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1–295.

[4] Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 80, 26.3.2010, p. 19–27.

A joint application has been introduced by the companies "Kerry Ingredients & Flavours" and "DSM Food Specialties B.V" for the re-evaluation, modification of the specifications and extension of use of the authorization of food enzyme β-fructofuranosidase (Invertase) from *Saccharomyces cerevisiae*.

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[5] implementing Regulation (EC) No 1331/2008, the Commission has verified that the application falls within the scope of the food enzyme Regulation and contains the relevant elements required under Chapter II of that Regulation.

1.1.2. Terms of Reference

The European Commission requests the European Safety Authority to carry out the safety assessment on the food enzyme β-fructofuranosidase from *Saccharomyces cerevisiae* in accordance with Article 17.3 of Regulation (EC) 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 β-fructofuranosidase from *S. cerevisiae*. The applicants have submitted three independent data packages corresponding to the former question number EFSA-Q-2015-00323. The current opinion addresses the food enzyme produced with strain INV submitted by DSM Food Specialties B.V., under a new question number (EFSA-Q-2021-00695).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme β-fructofuranosidase from *S. cerevisiae* (strain INV).

Additional information was requested from the applicant during the assessment process on 1 March 2022 and received on 1 August 2022 (see 'Documentation provided to EFSA').

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) and following the relevant existing guidance documents of EFSA Scientific Committee.

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 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	**β-Fructofuranosidase**
Systematic name	β-D-Fructofuranoside fructohydrolase
Synonyms	Invertase; saccharase; glucosucrase; β-fructosidase
IUBMB No	EC 3.2.1.26
CAS No	9001-57-4
EINECS No	232-615-7

β-Fructofuranosidases catalyse the hydrolysis of the terminal non-reducing β-D-fructofuranoside residues in β-D-fructofuranosides, releasing fructose. The enzyme under assessment is intended to be used in four food processes: manufacture of specialty carbohydrates (invert sugar syrups), baking processes, production of sucrose-based fermented beverages and confectionary processes.

[5] 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.

3.1. Source of the food enzyme

The β-fructofuranosidase is produced with the non-genetically modified yeast *Saccharomyces cerevisiae* strain INV, which is deposited at the Westerdijk Fungal Biodiversity Institute culture collection (CBS, the Netherlands) with the deposit number [redacted].[6]

The production strain was identified as *S. cerevisiae* [redacted].

The species *S. cerevisiae* is included in the list of organisms for which the qualified presumption of safety (QPS) may be applied, provided that the absence of resistance to antimycotics used for medical treatment of yeast infections is demonstrated in cases where viable cells are added to the food or feed chain (EFSA, 2007; EFSA BIOHAZ Panel, 2020; EFSA BIOHAZ Panel, 2021). The production strain was shown not to be present in the final food enzyme.[7] Therefore, the production strain was considered to qualify for the QPS approach to safety assessment.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004,[8] with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice (GMP).[9]

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 cells are lysed using [redacted] to aid the release of the enzyme from the cell wall. The solid biomass is then removed from the fermentation broth by filtration. The filtrate containing the enzyme is further purified and concentrated, including an ultrafiltration step in which the enzyme is retained, while most of the low molecular mass material passes the filtration membrane and is discarded.[10] The applicant provided information on the identity of the substances used to control the fermentation and in the subsequent downstream processing of the food enzyme.[11]

The 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

WGS analysis showed that the production strain produces two β-fructofuranosidases, which are single polypeptide chains. The first has [redacted] amino acids and a calculated molecular mass of the mature protein of [redacted] kDa; the second has [redacted] amino acids and a calculated molecular mass of the mature protein of [redacted] kDa.[12] The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. A consistent protein pattern was observed across all batches. The gels showed a broad protein band from [redacted] to [redacted] kDa, which is higher than the calculated molecular mass of the main variant. This can be explained by the fact that ß-fructofuranosidase is known to be heavily glycosylated (Sainz-Polo et al., 2013; Andjelkovic et al., 2020). No other enzyme activities were reported.[13]

The in-house determination of β-fructofuranosidase activity is based on hydrolysis of sucrose (reaction conditions: pH 4.5, 20°C, 30 min), measuring the release of monosaccharides spectrophotometrically. The enzyme activity is expressed in Sumner Unit/g (SU/g). One SU is defined as the quantity of enzyme which, under the conditions of the assay, will convert 1 mg of sucrose to glucose and fructose in 5 min.[14]

[6] Technical dossier/pg. 31/Annex 15.
[7] Additional data August 2022/Annex 1.
[8] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of food additives. OJ L 226, 25.6.2004, pp. 3–21.
[9] Technical dossier/pg. 33/Annex 5.
[10] Technical dossier/pg. 33-40/Annex 6.
[11] Technical dossier/Annex 7/Additional data August 2022.
[12] Technical dossier/pg. 25-26/Annex 13.
[13] Technical dossier/pg. 26–27.
[14] Technical dossier/pg. 26/Annex 2.

The food enzyme has a temperature optimum around 65°C (pH 4.5) and a pH optimum around pH 4.5 (30°C). Thermostability was tested after a pre-incubation of the food enzyme for 5 min and 30 min at different temperatures (pH 4.5). The activity decreased above 55°C, showing no residual activity above 70°C after 5 min of pre-incubation.[15]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three commercial batches (Table 1).[16] The mean total organic solids (TOS) of the three batches was 14.6% and the mean enzyme activity/TOS ratio was 679.7 SU/mg TOS.

Table 1: Composition of the food enzyme

Parameters	Unit	Batches		
		1	2	3
β-Fructofuranosidase activity	SU/g[(a)]	97,900	78,600	113,000
Protein	%	3.8	3.3	7.3
Ash	%	0.2	0.3	0.6
Water	%	82.3	89.6	83.3
Total organic solids (TOS)[(b)]	%	17.5	10.1	16.1
Activity/TOS	SU/mg TOS	559	778	702

(a): SU: Sumner Unit (see Section 3.3.1).
(b): TOS calculated as 100% - % water - % ash.

3.3.3. Purity

The lead content in the three batches was below 5 mg/kg,[17,18] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).

The food enzyme complies with the microbiological criteria for total coliforms, *Escherichia coli* and *Salmonella*, as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[17] No antimicrobial activity was detected in any of the three tested batches.[17]

The amount of [redacted] remaining in the food enzyme was measured in the three commercial batches by ^{1}H-nuclear magnetic resonance spectroscopy.[19] In one batch, the level was below the limit of detection (LoD), while in the other two, the levels were 14 and 17 mg/L. The highest amount of [redacted] found in the food enzyme (17 mg/L)[20] corresponds to an intake of 0.29 μg/person per day. This is three orders of magnitude lower than the estimated intake of [redacted] as a flavouring substance ([redacted] JECFA, 1998). The Panel considered this residual amount of [redacted] in the food enzyme of no safety concern.

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

3.3.4. Absence of viable cells

The absence of viable cells of the production strain was confirmed in three batches of the food enzyme [redacted]. No growth was observed. A positive control was included.

3.4. Toxicological data

As the production strain qualifies for the QPS approach to safety assessment and no issues of concern arising from the production process of the food enzyme were identified (see Sections 3.1, 3.2

[15] Technical dossier/pg. 27–29.
[16] Technical dossier/pg. 23/Annexes: 1, 2,3.
[17] Technical dossier/pg. 25/Annexes: 3, 4.
[18] LoD: Pb >2 mg/kg.
[19] Limit of quantification (LoQ) = 10 mg/L for [redacted]
[20] Additional data August 2022.

and 3.3), the Panel considered that no toxicological studies other than assessment of allergenicity were necessary.

3.4.1. Allergenicity

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

The potential allergenicity of the β-fructofuranosidase produced with the *S. cerevisiae* strain INV was assessed by comparing its amino acid sequence with those of known allergens according to the 'Scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms' (EFSA GMO Panel, 2010). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, one match was found.[21] The matching allergen was Sola l 2, a β-fructofuranosidase from *Solanum lycopersicum* (tomato), a known food allergen.

No information was available on oral and respiratory sensitisation or elicitation reactions of this β-fructofuranosidase.

The sequence homology of the β-fructofuranosidase produced with the *S. cerevisiae* strain INV with Sola l 2 indicates a potential cross-reactivity of the enzyme with the allergen from tomato. Allergic reactions to tomato occur, although they are not very frequent.

A study by Horner et al. (2008) reported reactivity to β-fructofuranosidase in individuals allergic to mould. However, several studies have shown that adults respiratorily sensitised may be able to ingest the corresponding allergen without acquiring clinical symptoms of food allergy (Brisman, 2002; Poulsen, 2004; Armentia et al., 2009).

[redacted], a known source of allergens, is present in the media fed to the microorganisms. However, during the fermentation process, it will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the fungal biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that no potentially allergenic residues are present in the food enzyme.

The Panel considered that, under the intended conditions of use, the risk of allergic reactions upon dietary exposure to this food enzyme, particularly in individuals sensitised to tomato, cannot be excluded. However, the likelihood of allergic reactions to the β-fructofuranosidase from *S. cerevisiae* strain INV is expected not to exceed the likelihood of allergic reactions to tomato. As the prevalence of allergic reactions to tomato is low, also the likelihood of such reaction to occur to the food enzyme is low.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in four food manufacturing processes at the recommended use levels summarised in Table 2.

Table 2: Intended uses and recommended use levels of the food enzyme as provided by the applicant[22]

Food manufacturing process[(a)]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[(b)]
Manufacture of speciality carbohydrates (invert sugar syrups)	Sucrose solution	88–**147**
Baking processes	Flour with or without added sucrose	0.3–**294**
Production of sucrose-based fermented beverages	Sucrose solution	74–**295**
Confectionary processes	Sucrose-containing filling	3–**29**

[21] Technical dossier/pg. 51-53/Annex 12.
[22] Additional data August 2022/Answer 4 and 5.

TOS: total organic solids.
(a): The description provided by the applicant has been harmonised by EFSA according to the 'EC working document describing the food processes in which food enzymes are intended to be used' – not yet published at the time of adoption of this opinion.
(b): The numbers in bold were used for calculations.

The addition of the food enzyme to sucrose solution results in the production of invert sugar syrup.[23] The β-fructofuranosidase hydrolyses sucrose to D-glucose and D-fructose, which enhances sweetness. Invert sugar syrup is a food product *per se* and is also used as a food ingredient in a variety of foods. The food enzyme remains in the syrups.

Sucrose is often used as a food ingredient to produce other foods. In these cases, the food enzyme is added to sucrose together with other food ingredients during the relevant food manufacturing processes. In baking processes, the food enzyme is added to flour with or without added sucrose[24] during dough preparation.[25] The enzyme also hydrolyses fructans in grains and flour from different cereals, which improves the digestibility of the products.[24] The food enzyme remains in the baked products.

The addition of the food enzyme to a sucrose solution, followed by fermentation, results in the production of alcoholic beverages.[26] The food enzyme remains in this type of flavoured alcoholic beverages (e.g. hard seltzers).[27]

In the manufacture of soft-centred confectionery, the food enzyme is added directly to the sucrose-containing fillings. The hydrolytic reaction of the β-fructofuranosidase can increase humectant properties of the sweet filling in confectionary products. The food enzyme remains active in this type of confectionery (e.g. candy).

Based on data provided on thermostability (see Section 3.3.1), it is expected that the β-fructofuranosidase will be inactivated by heat during all the intended food manufacturing processes, except for the confectionary processes.

3.5.2. Dietary exposure estimation

A list of food groups relevant for the estimation of the dietary exposure for this food enzyme–TOS were identified.[28]

Chronic exposure to the food enzyme–TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSA CEP Panel, 2021a). The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP Panel, 2021b). Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period (days) and normalised for body weight. This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only 1 day per subject were excluded and high-level exposure/intake was calculated for only those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011).

Table 3 provides an overview of the derived exposure estimates across all surveys. Detailed mean and 95th percentile exposure to the food enzyme–TOS per age class, country and survey, as well as the contribution from each FoodEx category to the total dietary exposure are reported in Appendix A – Tables 1 and 2. For the present assessment, food consumption data were available from 41 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). The highest dietary exposure was estimated to be 2.51 mg TOS/kg bw per day in toddlers at the 95th percentile.

[23] Technical dossier/p. 42.
[24] Additional data August 2022/Answer 4.
[25] Technical dossier/p. 43.
[26] Technical dossier/p. 44.
[27] Additional data August 2022/Answer 6.
[28] Additional data August 2022/Answer 7.

Table 3: Summary of estimated dietary exposure to food enzyme–TOS in six population groups

Population group	Estimated exposure (mg TOS/kg body weight per day)					
	Infants	**Toddlers**	**Children**	**Adolescents**	**Adults**	**The elderly**
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0.053–0.702 (12)	0.058–0.795 (15)	0.081–0.748 (19)	0.087–0.409 (21)	0.058–0.261 (22)	0.034–0.102 (23)
Min–max 95th percentile (number of surveys)	0.129–1.633 (9)	0.172–2.510 (14)	0.265–2.072 (19)	0.274–1.194 (20)	0.170–0.832 (22)	0.105–0.304 (21)

TOS: total organic solids.

3.5.3. Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the following sources of uncertainties have been considered and are summarised in Table 4.

Table 4: Qualitative evaluation of the influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction of impact
Model input data	
Consumption data: different methodologies/representativeness/ underreporting/misreporting/no portion size standard	+/–
Use of data from food consumption surveys of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Possible national differences in categorisation and classification of food	+/–
Model assumptions and factors	
FoodEx categories included in the exposure assessment assumed to always contain the food enzyme–TOS	+
Assumption that all sugars in the selected food groups are inverted sugar or syrups	+
Hard seltzers emerged in the recent 5 years and do not have a matching FoodEx code.	–
Exposure to food enzyme–TOS always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
Use of recipe fractions to disaggregate FoodEx categories	+/–
Use of technical factors in the exposure model	+/–

TOS: total organic solids.
+: Uncertainty with potential to cause overestimation of exposure.
–: Uncertainty with potential to cause underestimation of exposure.

The conservative approach applied to the exposure estimate to food enzyme–TOS, in particular assumptions made on the occurrence and use levels of this specific food enzyme, is likely to have led to an overestimation of the exposure.

3.6. Margin of exposure

Since no toxicological assessment was considered necessary by the Panel, the margin of exposure was not calculated.

4. Conclusion

Based on the data provided, the QPS status of the production strain and the absence of issues of concern arising from the production process, the Panel concluded that the food enzyme β-fructofuranosidase produced with the *Saccharomyces cerevisiae* strain INV does not give rise to safety concerns under the intended conditions of use.

5. Documentation as provided to EFSA

ß-Fructofuranosidase from *Saccharomyces cerevisiae*. November 2021. Submitted by DSM Food Specialties B.V.

Additional information. August 2022. Submitted by DSM Food Specialties B.V.

References

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

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

EFSA (European Food Safety Authority), 2006. Opinion of the Scientific Committee related to uncertainties in dietary exposure assessment. EFSA Journal 2006;5(1):438, 54 pp. https://doi.org/10.2903/j.efsa.2007.438

EFSA (European Food Safety Authority), 2007. Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA (2017–2019)Opinion of the Scientific Committee on a request from EFSA on the introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. EFSA Journal 2007;5(12):587, 16 pp. https://doi.org/10.2903/j.efsa.2007.587

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

EFSA (European Food Safety Authority), 2009b. Guidance of the Scientific Committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: general principles. EFSA Journal 2009;7(5):1051, 22 pp. https://doi.org/10.2903/j.efsa.2009.1051

EFSA (European Food Safety Authority), 2011. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011, 9(3) 2097, 34. https://doi.org/10.2903/j.efsa.2011.2097

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2020. Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA (2017–2019). EFSA Journal 2020;18(2):5966, 56 pp. https://doi.org/10.2903/j.efsa.2020.5966

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez- Ordonez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Hilbert F, Lindqvist R, Nauta M, Peixe L, Ru G, Simmons M, Skandamis P, Suffredini E, Cocconcelli PS, Fernandez Escamez PS, Prieto-Maradona M, Querol A, Sijtsma L, Suarez JE, Sundh I, Vlak J, Barizzone F, Hempen M and Herman L, 2021. Statement on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 14: suitability of taxonomic units notified to EFSA until March 2021. EFSA Journal 2021;19(7):6689, 41 pp. https://doi.org/10.2903/j.efsa.2021.6689

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré C, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mengelers M, Mortensen A, Rivière G, Steffensen I-L, Tlustos C, Van Loveren H, Vernis L, Zorn H, Glandorf B, Herman L, Aguilera J, Andryszkiewicz M, Gomes A, Kovalkovicova N, Liu Y, Rainieri S and Chesson A, 2021a. Scientific Guidance for the submission of dossiers on Food Enzymes. EFSA Journal 2021;19(10):6851, 37 pp. https://doi.org/10.2903/j.efsa.2021.6851

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré C, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mengelers M, Mortensen A, Rivière G, Steffensen I-L, Tlustos C, Van Loveren H, Vernis L, Zorn H, Liu Y and Chesson A, 2021b. Process-specific technical data used in exposure assessment of food enzymes. EFSA Journal 2021;19(12):7010, 38 pp. https://doi.org/10.2903/j.efsa.2021.7010

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2016. Panel statement on the exposure assessment of food enzymes. EFSA Journal 2016;14(11):4581, 9 pp. https://doi.org/10.2903/j.efsa.2016.4581 Available online: https://efsa.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.2903%2Fj.efsa.2016.4581&file=efs24581-sup-0001-Annex_B.pdf

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010. Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal 2010;8(7):1700, 168 pp. https://doi.org/10.2903/j.efsa.2010.1700

FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization), 2006. General specifications and considerations for enzyme preparations used in food processing in Compendium of food additive specifications. 67th meeting. FAO JECFA Monographs, 3, 63–67. Available online: http://www.fao.org/3/a-a0675e.pdf

Horner WE, Armstrong M, El-Dahr J, McCants M, Reese G, Kobernick AK and Lehrer SB, 2008. Prevalence of IgE reactivities in mold-allergic subjects to commercially available fungal enzymes. Allergy and Asthma Proceedings, 29(6), 629–635.

JECFA, 1998. Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 17-26 June 1997. WHO Technical Report Series, no. 884. Geneva.

Poulsen LK, 2004. Allergy assessment of foods or ingredients derived from biotechnology, gene-modified organisms, or novel food. Molecular Nutrition & Food Research, 48, 413–423.
Sainz-Polo MA, Ramírez-Escudero M, Lafraya A, González B, Marín-Navarro J, Polaina J and Sanz-Aparicio J, 2013. Three-dimensional structure of saccharomyces invertase. The Journal of Biological Chemistry, 288(14), 9755–9766.

Abbreviations

CAS	Chemical Abstracts Service
CBS	Westerdijk Fungal Biodiversity Institute culture collection
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LoD	limit of detection
LoQ	limit of quantification
QPS	qualified presumption of safety
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TOS	total organic solids
WGS	whole genome sequencing
WHO	World Health Organization

Appendix A – Dietary exposure estimates to the food enzyme–TOS in details

Information provided in this appendix is shown in an excel file (downloadable at https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2023.7833#support-information-section).

The file contains two sheets, corresponding to two tables.

Table 1: Average and 95th percentile exposure to the food enzyme–TOS per age class, country and survey.

Table 2: Contribution of food categories to the dietary exposure to the food enzyme–TOS per age class, country and survey.

Appendix B – Population groups considered for the exposure assessment

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From 12 weeks on up to and including 11 months of age	Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia, spain
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Netherlands, Portugal, Slovenia, Spain
Children	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
The elderly[(a)]	From 65 years of age and older	Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden

(a): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011).