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Supporting document

Risk and technical assessment – Application A1358

Glucoamylase from *Trichoderma reesei* (gene donor: *Aspergillus fumigatus*) for use as a processing aid

Executive summary

Danisco Australia Pty Ltd submitted an application to permit the use of glucoamylase (EC 3.2.1.3) from *Trichoderma reesei* containing the glucoamylase gene from *Aspergillus fumigatus*. The glucoamylase is intended for use as a processing aid in brewing, potable alcohol production and starch processing in accordance with Good Manufacturing Practice (GMP).

Glucoamylase converts starch substrates to fermentable sugar in brewing and potable alcohol production, and by saccharifying liquefied corn starch for the manufacture of high glucose syrups and corn sweeteners.

Glucoamylase performs its technological purpose during the production of the nominated foods, after which it is inactivated, and does not perform a technological purpose in the final food. It therefore functions as a processing aid for the purposes of the Australia New Zealand Food Standards Code (the Code).

There are relevant identity and purity specifications for the enzyme in the Code, and the applicant provided evidence that the enzyme meets these specifications.

No public health or safety concerns related to the use of the applicant's *T. reesei* strain as a production organism for glucoamylase were identified in FSANZ's microbiological assessment. Analysis of the production strain confirmed the presence and stability of the inserted DNA.

No significant homology between glucoamylase from *T. reesei* and known toxins or food allergens was identified. The enzyme concentrate was not genotoxic in vitro, and no adverse effects were observed in a 90-day oral toxicity study in rats. The no observed adverse effect level (NOAEL) was 1000 mg total organic solids (TOS)/kg bw/day, the highest dose tested.

A dietary exposure assessment was not conducted for this application because the concentrations requested in A1358 were lower than those in the dietary exposure scenario assessed for a glucoamylase enzyme in Application A1291. A theoretical maximum daily intake (TMDI) of 6.15 mg TOS/kg bw/day was calculated in the evaluation for A1291.

A comparison of the NOAEL for the enzyme concentrate and the TMDI results in a margin of exposure (MOE) of approximately 200. Based on the reviewed information it is concluded

that in the absence of any identifiable hazard an acceptable daily intake (ADI) 'not specified' is appropriate.

FSANZ concludes there are no public health and safety concerns from the proposed use of glucoamylase produced by *T. reesei* containing the glucoamylase gene from *Aspergillus fumigatus*.

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1. Introduction

Danisco Australia Pty Ltd has applied to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of the enzyme glucoamylase (EC 3.2.1.3) as a processing aid in brewing, the production of potable alcohol and starch processing. The enzyme is sourced from a genetically modified (GM) strain of *Trichoderma reesei* containing the glucoamylase gene from *Aspergillus fumigatus*.

The enzyme will be used to convert starch to fermentable sugar in brewing and potable alcohol production, and to saccharify liquefied corn starch for the manufacture of high glucose syrups and corn sweeteners. It is intended to be used at the minimum level required to achieve the desired effect, in accordance with the principles of Good Manufacturing Practice (GMP)¹.

Schedule 18 of the Code includes permission for several glucoamylase enzymes produced by *T. reesei* containing the glucoamylase gene from *T. reesei* using a variety of donors but not including *A. fumigatus*. Glucoamylase produced by *T. reesei* containing the glucoamylase gene from *A. fumigatus* needs pre-market assessment before permission can be given for its use as a processing aid.

1.1 Objectives of the assessment

The objectives of this risk and technical assessment were to:

- determine whether the proposed purpose is solely technological and that the enzyme achieves its technological purpose in the quantity and form proposed to be used as a food processing aid
- evaluate potential public health and safety concerns that may arise from the use of this enzyme by considering the:
 - safety and history of use of the production microorganism
 - safety of the enzyme preparation.

Some information relevant to the assessment is deemed confidential commercial information (CCI) under section 114 of the *Food Standards Australia New Zealand Act 1991* (the FSANZ Act) and has been evaluated by FSANZ but cannot be disclosed in this report.

2 Food technology assessment

2.1 Identity of the enzyme

The applicant provided relevant information regarding the identity of the enzyme, and this has been verified using the IUBMB² enzyme nomenclature reference database (IUBMB,

¹ GMP is defined in section 1.1.2—2 of the Code as follows: *with respect to the addition of substances used as food additives and substances used as processing aids to food, means the practice of:*
(a) *limiting the amount of substance that is added to food to the lowest possible level necessary to accomplish its desired effect; and*
(b) *to the extent reasonably possible, reducing the amount of the substance or its derivatives that:*
(i) *remains as a *component of the food as a result of its use in the manufacture, processing or packaging;*
and
(ii) *is not intended to accomplish any physical or other technical effect in the food itself;*

² International Union of Biochemistry and Molecular Biology

2018). Details of the identity of the enzyme are provided below.

Accepted IUBMB name:	Glucan 1,4- α -glucosidase
Systematic name:	4- α -D-Glucan Glucohydrolase
Other names:	Glucoamylase; Amyloglucosidase; γ -amylase; Lysosomal α -Glucosidase; Acid maltase; Exo-1,4- α -Glucosidase; Glucose Amylase; γ -1,4-Glucan Glucohydrolase; Acid Maltase; 1,4- α -D-Glucan Glucohydrolase
IUBMB enzyme nomenclature:	EC 3.2.1.3
CAS number ³ :	9032-08-0
Reaction:	Hydrolysis of terminal (1 $\rightarrow$ 4)-linked α -D-glucose residues successively from non-reducing ends of the chains with release of β -D-glucose

2.2 Manufacturing process

2.2.1 Production of the enzyme

Enzymes produced from microorganisms are typically produced by controlled fermentation followed by removal of the production microorganism, purification and concentration of the enzyme. Final standardisation with stabilisers, preservatives, carriers, diluents, and other approved food-grade constituents is carried out after the purification and concentration steps. The formulated enzymes are referred to as enzyme preparations, which, depending upon the application in food, may be a liquid, semi-liquid or dried product. Enzyme preparations may contain either one major active enzyme that catalyses a specific reaction during food processing or two or more active enzymes that catalyse different reactions (FAO/WHO 2020).

2.2.2 Specifications for identity and purity

There are international general specifications for enzyme preparations used in the production of food. These have been established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in its Compendium of Food Additive Specifications (FAO JECFA Monographs 26 (2021)), explicitly FAO/WHO (2006) and in the Food Chemicals Codex (FCC 2022), referenced in section 3—2 of Schedule 3 of the Code.

Enzymes used as a processing aid need to meet either of these specifications, or a relevant specification in section S3—3 of Schedule 3. In addition, under JECFA, enzyme preparations must meet the specifications criteria contained in the individual monographs. In the case of glucoamylase, there is no individual monograph. Schedule 3 of the Code also includes specifications for arsenic and heavy metals (section S3—4) if they are not already detailed within specifications in sections S3—2 or S3—3.

The applicant provided the results of analysis of 3 different batches of their enzyme concentrate. Table 1 provides a comparison of the results of those analyses with international specifications established by JECFA and Food Chemicals Codex, as well as

³ Chemical Abstracts Service

those in the Code (as applicable). Based on those results, the enzyme met all relevant specifications.

Table 1 Analysis of manufacturer's glucoamylase enzyme concentrate compared to JECFA, Food Chemicals Codex, and Code specifications for enzymes

Test parameters	Danisco Australia Pty Ltd test results	Specifications		
		JECFA	Food Chemicals Codex	The Code - section S3—4
Lead (mg/kg)	<0.01	≤5	≤5	≤2
Arsenic (mg/kg)	0.02	-	-	≤1
Cadmium (mg/kg)	<0.001	-	-	≤1
Mercury (mg/kg)	<0.005	-	-	≤1
Coliforms (cfu/g)	<1	≤30	≤30	-
<i>Salmonella</i> (in 25 g)	Absent	Absent	Negative	-
<i>Escherichia coli</i> (in 25 g)	Absent	Absent	-	-
Antimicrobial activity	Absent	Absent	-	-

cfu = colony forming units

The specification for the enzyme concentrate used by the manufacturer (as provided in Annex 2 of the application) includes a test for the absence of the production strain (Annex 5). The enzyme, however, is a biological isolate of variable composition, containing the enzyme protein, as well as organic and inorganic material derived from the microorganism and fermentation process. The applicant stated the enzyme preparation is used at the minimum level required to achieve the desired effect, in accordance with the principles of GMP. The highest use level of the enzyme given is for potable alcohol production, at 0.4 Glucoamylase units⁴ (GAU) per gram of cereal (grist). This is equivalent to 229.6 mg total organic solids (TOS) per kilogram of raw material. TOS encompasses the enzyme component and other organic material originating from the production organism and the manufacturing process, while excluding intentionally added formulation ingredients.

2.3 Technological purpose

Glucoamylase is intended for use as a processing aid in brewing, the production of potable alcohol and starch processing. Glucoamylase will be used to convert starch to fermentable sugar in brewing and potable alcohol production, and to saccharify liquefied corn starch for the manufacture of high glucose syrups and corn sweeteners. The enzyme is intended to be used in starch processing where it may confer benefits including enhancing fermentability and sweetness by breaking down starch, leading to more consistent product quality. The applicant requested use of the enzyme at GMP levels.

As identified by the IUBMB (section 2.1, above), glucoamylase hydrolyses terminal (1→4)-linked α-D-glucose residues successively from non-reducing ends of the chains with release of β-D-glucose. Most forms of the enzyme can also rapidly hydrolyse (1→6)-α-D-glucosidic bonds when the next bond in the sequence is (1→4), and some preparations of this enzyme hydrolyse both (1→6)- and (1→3)-α-D-glucosidic bonds in other polysaccharides. For a representation of the hydrolysis catalysed by glucoamylase, refer to its record in the enzyme

⁴ Where one GAU is the amount of enzyme which will liberate one gram of sugar as glucose per hour under the conditions of the assay

database BRENDA⁵ (Chang et al 2021).

The technological purpose as stated by the applicant of glucoamylase is the conversion of starch to fermentable sugar in brewing and potable alcohol production, and in saccharification of liquefied corn starch for the manufacture of high glucose syrups and corn sweeteners is consistent with the typical function of glucoamylase and is supported by scientific literature, which indicates that this enzyme is principally responsible for the technological purpose (Kumar and Satyanarayana 2009).

Glucoamylase hydrolyses starch (saccharification) to release glucose to produce various products such as alcohol (ethanol), amino and organic acids. End products can be further processed to produce glucose syrups and other starch hydrolysates (Kumar and Satyanarayana 2009). Starch hydrolysates are a combination of polyhydric alcohols and can include maltitol and sorbitol (Modderman 1993).

In brewing, the enzyme can be added during fermentation to metabolise dextrins (residual carbohydrates) converting them into fermentable sugars, which affects alcohol levels (Blanco et al. 2014). In starch hydrolysis to produce glucose syrups, this enzyme is beneficial due to it being able to withstand higher temperatures, reducing the risk of microbial growth.

The applicant provided information on the physical and chemical properties of the enzyme concentrate (Table 2).

Table 2 Glucoamylase enzyme concentrate physical/chemical properties

Physical/chemical properties of commercial enzyme preparation	
Enzyme activity	Enzyme activity is based on the ability of glucoamylase enzyme to catalyse the hydrolysis of p-nitrophenylalapha-D-glucopyranoside (PNPG) to glucose and p-nitrophenol. Activity is defined in Glucoamylase units (GAU).
Appearance	Off white powder
Temperature range	Optimum 65-75°C
Temperature stability	Inactivated at 76°C for 30 minutes
pH range and optimum	Max activity within range 4.5-5.5

Following the hydrolysis reaction, residual proteins will be removed through washing and/or filtration. In starch processing the enzyme undergoes a series of purification steps (filtration, carbon treatment and ion exchange) which removes residual protein. In brewing, the enzyme is removed in post boiling clarification processes. Residual enzyme activity would potentially adversely affect beer quality and consistency, so it is common practice to assay for residual enzyme activity after beer pasteurisation. In potable alcohol production, after fermentation, solids are separated from the fermentation slurry, and any enzyme protein precipitate will be removed. The liquids are then distilled at approximately 82°C and boiled at 149°C during which the enzyme will be denatured. Information provided by the applicant states that the enzyme is inactivated at 76°C. The literature surrounding enzyme activity at higher temperatures (above 60°C) supports this (Ezugwu, Eze and Chilaka 2015). Glucoamylase will be used as a processing aid and is subsequently denatured, so it is not present in the final food in an active form and has no ongoing technological function.

⁵ [Information on EC 3.2.1.3 - glucan 1,4-alpha-glucosidase - BRENDA Enzyme Database](#)

2.4 Food technology conclusion

FSANZ concludes that glucoamylase has been demonstrated to perform its typical function of the conversion of starch to fermentable sugar in brewing and potable alcohol production, and to saccharify liquefied corn starch for the manufacture of high glucose syrups and corn sweeteners.

FSANZ concludes that the evidence presented to support its proposed use provides adequate assurance that the use of the enzyme, in the quantity and form proposed to be used (which must be consistent with GMP), is technologically justified.

Glucoamylase performs its technological purpose during the production of the nominated foods, after which it is inactivated, and is not performing a technological purpose in the final food. It is therefore functioning as a processing aid for the purposes of the Code.

There are relevant identity and purity specifications for the enzyme in the Code, and the applicant provided evidence that the enzyme meets these specifications.

3 Safety assessment

This safety assessment aims to evaluate potential public health and safety concerns arising from using the glucoamylase enzyme associated with a genetically modified strain of *T. reesei* as a processing aid.

Some information relevant to this section is deemed CCI under the FSANZ Act) and has been evaluated by FSANZ but cannot be disclosed in this report..

3.1 Source microorganism

FSANZ has previously assessed the safety of *T. reesei* as the source organism for a range of processing aids listed in Schedule 18. Several enzymes produced by *T. reesei* QM6a have Generally Recognized as Safe (GRAS) status with the Food and Drug Administration (FDA) or the FDA had no questions about the GRAS conclusions contained in GRAS submissions (US EPA 2012).

T. reesei is a biosafety level 1, common, hypercellulolytic, soil fungus that was initially isolated from deteriorating canvas made from cellulosic material. Strain QM6a is the original wild-type strain for *T. reesei* and has been registered with the American Type Culture Collection as ATCC13631 (Olempska-Beer et al. 2006). Strain QM6a is the parental strain of the majority of *T. reesei* industrial production strains (Nevalainen et al. 1994).

T. reesei has a history of safe use in industrial-scale enzyme production (Nevalainen et al. 1994, Blumenthal 2004, Nevalainen and Peterson 2014, Paloheimo et al. 2016, Frisvad et al. 2018). Food enzymes derived from *T. reesei* strains (including recombinant *T. reesei* strains) have been evaluated by JECFA and many countries which regulate the use of food enzymes, such as Australia and New Zealand, USA, France, Denmark and Canada.

T. reesei QM6a derived strains are non-pathogenic, not known to possess any virulence factors associated with either colonisation or disease, and do not present any human toxicity concerns (US EPA 2012). Although some *Trichoderma* species can produce various mycotoxins and antifungal metabolites, several review papers support the safety of *T. reesei* QM6a strains concluding that there was no production of known mycotoxins or antibiotics under conditions used for enzyme production (Nevalainen et al. 1994, Kubicek et al. 2007, Peterson and Nevalainen 2012, Frisvad et al. 2018). *T. reesei* QM6a strains are known to

produce the peptaibol antibiotic paracelsin, but industry-standard submerged fermentation conditions are not linked to the production of paracelsin (US EPA 2012).

The applicant provided data that adequately demonstrates the production strain's identity as a derivative of *T. reesei* QM6a. Microbiological data was provided to FSANZ confirming the absence of the production organism and mycotoxins in the final enzyme preparation.

A. fumigatus was the source organism for the glucoamylase gene inserted into the production strain (see section 3.2 for further details). While *A. fumigatus* is a known pathogen, the inserted glucoamylase gene is well-characterised, with no known role in pathogenicity.

No public health or safety concerns related to the use of the applicant's *T. reesei* production strain as a production organism for glucoamylase were identified in FSANZ's microbiological assessment.

3.2 Characterisation of the genetic modification to the production strain

3.2.1 Description of the DNA to be introduced and the method of transformation

The gene encoding the glucoamylase enzyme was derived from *A. fumigatus*. Data provided by the applicant and analysed by FSANZ confirmed the identity of the glucoamylase enzyme.

An expression cassette containing the glucoamylase gene was introduced into the genome of the host *T. reesei* using standard molecular biology techniques. The glucoamylase gene was placed under the control of the native *T. reesei* *cbh1* promoter and terminator. The native *T. reesei* *pyr2* gene, encoding orotate phosphoribosyltransferase, was used as a selectable marker to enable selection of positive transformants.

3.2.2 Characterisation of the inserted DNA

Southern blot analysis confirmed the presence of the expression cassette in the genome of the production strain. The analysis also confirmed that no antibiotic resistance marker genes or other bacterial vector DNA were present in the final production strain.

3.2.3 Stability of the introduced DNA

Whole genome sequencing data was provided to demonstrate stability of the inserted gene in the production strain. DNA extracted from cultures after prolonged fermentation and stock culture prior to fermentation as a control were analysed. This data confirmed the glucoamylase gene is expressed stably over multiple generations.

3.3 Safety of the enzyme

3.3.1 History of safe use

Several glucoamylase enzymes from other sources are already permitted for use as processing aids in both S18—4 (Permitted enzymes) and S18—9 (Permitted processing aids – various technological purposes).

The applicant's glucoamylase enzyme has been determined by a panel of experts to be generally recognized as safe (GRAS) in the USA and has also been approved for use in several countries. The countries where approval has been granted was provided as CCI. No

adverse events have been reported since the enzyme has been in commercial use in these countries.

3.3.2 Bioinformatic assessment of homology with known toxins

The applicant conducted a BLAST search for homology of the glucoamylase amino acid sequence with known toxins using the UniProt annotated Protein Knowledge database⁶. A threshold E-value⁷ of 1 was used. Most matches were cyclomaltodextrin glucanotransferases and glucoamylases, and none was annotated as a toxin or venom. Based on this assessment the glucoamylase sequence does not share homology with a known toxin or venom.

3.3.3 Toxicology data

The applicant submitted reports of two genotoxicity studies of the glucoamylase enzyme concentrate as well as a 90-day oral toxicity study in rats. The test items used in these studies are representative of the enzyme concentrate used to formulate the final commercial enzyme preparations.

Short-term toxicity

90-day oral toxicity study in rats (Korea Institute of Technology 2020) Regulatory status: GLP; conducted in accordance with OECD Test Guideline (TG) 408

Glucoamylase was administered by oral gavage to Sprague-Dawley rats (10/sex/group) at doses of 0, 250, 500 and 1000 mg TOS/kg bw/day for 90 days. Water was used as the vehicle/control. Clinical signs were monitored throughout the study. Body weights and food consumption were recorded weekly. Ophthalmological evaluations were performed on all animals before the start of treatment and again during the final week of the study. An abbreviated neurobehavioural test battery, consisting of selected functional observation battery assessments and motor activity was conducted on all animals before dosing and in the final week. Blood samples for thyroid hormone analysis were collected from all animals on the day of termination, and blood and urine samples were collected for haematology, coagulation, clinical chemistry and urinalysis parameters. At the end of the study animals were killed and examined macroscopically, and selected organs were weighed. Oestrus cycle examinations were performed on all females. Tissues from the control and high dose groups were examined microscopically.

No mortality, morbidity or clinical signs of toxicity were observed during the study, and there were no treatment-related changes in body weight, body weight gain or food consumption. There were no treatment-related adverse effects on any of the parameters evaluated.

The no observed adverse effect level (NOAEL) in this study was 1000 mg TOS/kg bw/day, the highest dose tested.

Genotoxicity

Genotoxicity studies with the glucoamylase enzyme concentrate comprised a bacterial reverse mutation test and an *in vitro* mammalian cell micronucleus test. These studies were GLP compliant and conducted according to OECD Test Guidelines. The positive controls

⁶ <https://www.uniprot.org/>

⁷ In BLAST (Basic Local Alignment Search Tool), the E-value or Expect value is a key metric used to assess the degree of similarity of the query protein to its corresponding matches in a database. The size of the E-value is inversely related to the degree of similarity, i.e. lower E-values indicate that the match is less likely to have occurred by chance.

produced the expected responses.

As summarised in Table 3, glucoamylase showed no evidence of mutagenicity, clastogenicity or aneugenicity in the presence or absence of metabolic activation.

Table 3: Genotoxicity studies with glucoamylase

Test	Test object	Concentration	Results	Reference
Bacterial reverse mutation test (OECD TG 471); treat and plate method	Salmonella typhimurium strains TA98, TA100, TA1535 & TA1537; Escherichia coli strain WP2 <i>uvrA</i>	Test I: 1.5 – 5000 µg/plate Test II: 50 – 5000 µg/plate	Negative ± S9	BioReliance (2020)
In vitro mammalian micronucleus test (OECD TG 487)	Human peripheral blood lymphocytes	Phase I (4 h exposure ± S9): 1250 – 5000 µg TOS/mL Phase II (24h exposure – S9): 312.5 – 2500 µg TOS/mL ^a	Negative ± S9	Jai Research Foundation (2024)

^a Cytotoxicity (replicative index of 24.03%) was observed at 5000 µg TOS/mL following 24 h incubation in the absence of metabolic activation; this concentration was therefore not scored for micronuclei.

3.3.4 Potential for allergenicity

Potential allergenicity of glucoamylase was assessed by comparing its amino acid sequence with those of known allergens. The following searches were conducted by the applicant using the AllergenOnline database v23 (30 January 2025):

- Full length alignments using an E-value of < 0.1
- > 35% identity over 80-amino acid stretches
- Exact matches over 8 contiguous amino acids

One sequence match was identified in all three searches, however the observed match is not listed as a food allergen. A literature search found reports of glucoamylase enzymes being associated with respiratory allergy (e.g. Baker's asthma; Quirce et al 2002), but no reports of food allergy were identified. In addition, studies have shown that individuals with respiratory allergies including Baker's asthma can safely consume the allergenic proteins (Armentia 2009; Poulsen 2004; Ramirez & Bahna 2009).

Based on the available evidence, glucoamylase is unlikely to pose a food allergenicity concern.

3.3.5 Assessments by other regulatory agencies

As noted in section 3.3.1, the applicant's glucoamylase enzyme has been determined by a panel of experts to be generally recognized as safe (GRAS) in the USA, and has been approved by several overseas authorities. Safety assessments from these agencies are not available to FSANZ.

4 Dietary exposure assessment

A dietary exposure assessment was not conducted for this application because the budget

method calculation conducted for Application A1291 considered the worst-case exposure scenario to glucoamylase which would cover uses outlined in Application A1358. In summary, A1291 included a higher glucoamylase concentration in all foods (590 mg TOS/kg in raw material) and beverages (821 mg TOS/kg in raw material) than the concentration requested for production of gluten, starch, potable alcohol, and beer in Application A1358 (91 mg TOS/kg raw material for foods; 91–230 mg TOS/kg raw material for beverages) and proportions of raw material in the final foods equal to or higher than noted in A1358 (FSANZ 2024). No exposure concerns were identified with the higher concentrations in Application A1291.

5 Discussion

The use of glucoamylase as a processing aid in brewing and potable alcohol production, and to saccharify liquefied corn starch for the manufacture of high glucose syrups and corn sweeteners is consistent with the typical function of the enzyme.

The enzyme is intended to be used in starch processing where it may confer benefits including enhancing fermentability and sweetness by breaking down starch, leading to more consistent product quality.

Glucoamylase is functioning as a processing aid for the purposes of the Code and does not perform a technological purpose in the food for sale. The available evidence presented to support its proposed use provides adequate assurance that the use of the enzyme, in the quantity and form proposed to be used (which must be consistent with GMP), is technologically justified.

There are relevant identity and purity specifications for the enzyme in the Code, and the applicant has provided evidence that the enzyme meets these specifications.

No public health or safety concerns related to the use of the applicant's *T. reesei* production strain as a production organism for glucoamylase were identified in FSANZ's microbiological assessment. Analysis of the production strain confirmed the presence and stability of the inserted DNA.

No significant homology between glucoamylase from *T. reesei* and known toxins or food allergens was identified. The enzyme was not genotoxic in vitro, and no adverse effects were observed in a 90-day oral toxicity study in rats. The NOAEL was 1000 mg TOS/kg bw/day, the highest dose tested in this study.

A dietary exposure assessment was not performed, however a worst-case exposure scenario for glucoamylase (from *Aspergillus niger*) was evaluated for Application A1291. A theoretical maximum daily intake (TMDI) of 6.15 mg TOS/kg bw/day was calculated in the evaluation for A1291. A comparison of the NOAEL for glucoamylase B and the TMDI results in a margin of exposure (MOE) of approximately 200. Based on the reviewed information it is concluded that in the absence of any identifiable hazard an acceptable daily intake (ADI) 'not specified' is appropriate.

FSANZ concludes there are no public health and safety concerns from the proposed use of glucoamylase B.

6 References

Armentia A, Díaz-Perales A, Castrodeza J, Dueñas-Laita A, Palacin A, Fernández S. (2009) Why can patients with baker's asthma tolerate wheat flour ingestion? Is wheat pollen allergy relevant? *Allergol*

BioReliance (2020) [REDACTED] Bacterial Reverse Mutation Assay BioReliance, Rockville, MD, USA

Blanco CA, Caballero I, Barrios R, Rojas A (2014) Innovations in the brewing industry: Light beer. *International Journal of Food Sciences and Nutrition* 65:655–60

Blumenthal CZ (2004) Production of toxic metabolites in *Aspergillus niger*, *Aspergillus oryzae*, and *Trichoderma reesei*:: justification of mycotoxin testing in food grade enzyme preparations derived from the three fungi. *Regulatory Toxicology and Pharmacology*, 39(2):214-228.doi:10.1016/j.yrtph.2003.09.002

Chang A., Jeske L., Ulbrich S., Hofmann J., Koblit J., Schomburg I., Neumann-Schaal M., Jahn D., Schomburg D. (2021) BRENDA, the ELIXIR core data resource in 2021: new developments and updates, *Nucleic Acids Res.*, 49: D498-D508.

Ezugwu AL, Eze SOO and Chilaka FC (2015) A study of the optimal conditions of glucoamylases obtained from *Aspergillus niger* using amylopectin from cassava starch as carbon source. *African Journal of Biotechnology* 14(36):2693-2702

FAO/WHO (2020) Environmental Health Criteria 240. Principles and Methods for the Risk Assessment of Chemicals in Food. Chapter 9.1.4: Processing aids. Second Edition 2020. WHO, Geneva. [Principles and methods for the risk assessment of chemicals in food \(who.int\)](https://www.who.int/publications/m/item/principles-and-methods-for-the-risk-assessment-of-chemicals-in-food)

Frisvad JC, Møller LL, Larsen TO, Kumar R, Arnau J (2018) Safety of the fungal workhorses of industrial biotechnology: update on the mycotoxin and secondary metabolite potential of *Aspergillus niger*, *Aspergillus oryzae*, and *Trichoderma reesei*, *Applied Microbiology and Biotechnology* 102(22):9481-9515

FSANZ (2024) Application A1291 – Glucoamylase from GM *Aspergillus niger* (gene donor: *Gloeophyllum sepiarium*) as a processing aid. Approval Report. Supporting Document 1: Risk Assessment (at Approval). Report prepared by Food Standards Australia New Zealand, Canberra. https://www.foodstandards.gov.au/sites/default/files/2024-11/A1291%20Supporting%20document_approval_0.pdf. Accessed 26 July 2026.

IUBMB (2018). EC 3.2.1.3 <https://iubmb.qmul.ac.uk/enzyme/EC3/2/1/3.html> Accessed 11 August 2026

Jai Research Foundation (2024) DP-Nzh120: In vitro mammalian cell micronucleus test in human peripheral blood lymphocytes. Jai Research Foundation, Gujarat, India.

Korea Institute of Toxicology (2020) [REDACTED] A 90-day repeated dose toxicity study administered by oral gavage in Sprague-Dawley rats. Korea Institute of Toxicology, KRICT, Daejeon 34114, Republic of Korea.

Kubicek CP, Komoř-Zelazowska M, Sándor E, Druzhinina IS (2007) Facts and Challenges in the Understanding of Biosynthesis of Peptaibols by *Trichoderma*. *Chemistry and Biodiversity* 4(6):1068-1082

Kumar, P and Satyanarayana, T. (2009). Microbial glycoamylases; characteristics and applications. *Critical Reviews in Biotechnology*. 29(3): 225-255.

Modderman JP (1993) Safety assessment of hydrogenated starch hydrolysates. *Regulatory Toxicology and Pharmacology*. 18(1):80-114

Nevalainen H and Peterson R (2014) Making recombinant proteins in filamentous fungi - are we expecting too much? *Frontiers of Microbiology*, 5:e75

Nevalainen H, Suominen P, Taimisto K (1994) Minireview. On the Safety of *Trichoderma Reesei*, *Journal of Biotechnology* 37:193-200

Olempska-Beer ZS, Merker RI, Ditto MD and DiNovi MJ (2006) Food-processing enzymes from recombinant microorganisms – A review. *Regulatory Toxicology and Pharmacology* 45:144-158

Paloheimo M, Haarmann T, Mäkinen S and Vehmaanperä J (2016) Production of industrial enzymes in *Trichoderma reesei*. In: Schmoll M, Dattenböck C (Eds) *Gene expression systems in Fungi: Advancements and Applications*, Springer International Publishing, Switzerland, p.23-57

Peterson R and Nevalainen H (2012) *Trichoderma reesei* RUT-C30-thirty years of strain improvement. *Microbiology*, 158: 58-68

Poulsen LK. (2004) Allergy assessment of foods or ingredients derived from biotechnology, gene-modified organisms, or novel foods. *Mol Nutr Food Res*. 48(6):413-23

Ramirez DA Jr, Bahna SL (2009) Food hypersensitivity by inhalation. *Clinical and Molecular Allergy* 7: 4

US EPA (2012) 40 CFR Part 725 Microorganisms; General Exemptions From Reporting Requirements; Revisions to Recipient Organisms Eligible for Tier I and Tier II Exemptions <https://www.federalregister.gov/documents/2020/03/10/2020-04746/microorganisms-general-exemptions-from-reporting-requirements-revisions-to-recipient-organisms>. Accessed 10 June 2026