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Supporting document

Risk and technical assessment – A1291

Glucoamylase from GM *Aspergillus niger* (gene donor: *Gloeophyllum sepiarium*) as a processing aid

Executive summary

Food Standards Australia New Zealand (FSANZ) received an application from Novozymes, Australia Pty Ltd to vary the Australia New Zealand Food Standards Code (the Code) to permit the use of a protein engineered variant of glucoamylase from genetically modified (GM) *Aspergillus niger* as a processing aid. This glucoamylase will be used in baking, brewing and starch processing to manufacture glucose syrups and other starch hydrolysates. Glucoamylase is derived by submerged fermentation of *A. niger* containing the glucoamylase gene from *Gloeophyllum sepiarium*.

The proposed use of glucoamylase as an enzyme processing aid in the quantity and form proposed is consistent with its typical function of hydrolysing starch. The enzyme performs its technological purpose during the production of the nominated foods and is not performing a technological purpose in the final food. The enzyme meets relevant identity and purity specifications in the Code.

There are no safety concerns from the use of glucoamylase from a GM strain of *A. niger* containing the glucoamylase gene from *G. sepiarium*. Glucoamylase from other sources has a long history of safe use in food. The production organism is neither pathogenic nor toxigenic. Analysis of the GM production strain confirmed the presence and stability of the inserted DNA.

A no observed adverse effect level (NOAEL) of 1070.2 mg total organic solids (TOS)/kg body weight (bw)/day was identified in a 13-week oral toxicity study in rats. The theoretical maximum daily intake (TMDI) of the Glucoamylase from *A. niger* enzyme was calculated to be 6.15 mg TOS/kg bw. A comparison of the NOAEL and the TMDI results in a Margin of Exposure (MOE) of approximately 200. Based on the reviewed data it is concluded that in the absence of any identifiable hazard an Acceptable Daily Intake (ADI) 'not specified' is appropriate. FSANZ concludes that there are no public health and safety concerns.

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1 Introduction

Novozymes 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). This enzyme is sourced from a genetically modified (GM) strain of *Aspergillus niger* containing a protein engineered variant of the glucoamylase gene from *Gloeophyllum sepiarium*. The production strain of *A. niger* used by the applicant was developed from the BO-1 cell lineage.

The enzyme is intended to be used as a processing aid in brewing processes, distilled alcohol production, starch processing for glucose syrup production, other starch hydrolysates, and baking processes where glucoamylase degrades starch into D-glucose. The enzyme will be used in accordance with the principles of Good Manufacturing Practice (GMP)[1], at the minimum level required to achieve the desired effect.

1.1 Objectives of the Assessment

The objectives of this risk and technical assessment were to:

- determine whether the proposed purpose is a solely technological purpose, and that the enzyme achieves its technological purpose as a processing aid in the quantity and form proposed to be used
- evaluate potential public health and safety concerns that may arise from the use of this food enzyme produced by a GM microorganism, by considering the:
  - safety and history of use of the host and gene donor organisms
  - characterisation of the genetic modification(s),
  - safety of the enzyme.

[1] GMP is defined in the Standard 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 Food Technology Assessment

2.1 Specifications for identity and purity

2.1.1 Identity

The applicant provided relevant information regarding the identity of the enzyme, and this has been verified using the IUBMB[2] enzyme nomenclature reference database (McDonald *et al.* 2009). Details of the identity of the enzyme are provided in Table 1.

Table 1 Identity

Systematic name	4-α-D-glucan glucohydrolase
Accepted IUBMB name:	glucan 1,4-alpha-glucosidase
Common 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[3] registry number:	9032-08-0
Reactions	Hydrolysis of terminal (1→4)-linked α-D-glucose residues successively from non-reducing ends of the chains with release of β-D-glucose.

The hydrolysis reaction catalysed by glucoamylase is shown in the database BRENDA[4] (Chang et al. 2021).

2.1.2 Purity and specifications

There are international general specifications for enzyme preparations used in the production of food. The Joint FAO have established these/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). Enzymes used as a processing aid need to meet either of these specifications. 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.[5]

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 three different batches of their glucoamylase. Table 2 provides a comparison of the results of those analyses with international specifications established by JECFA and Food Chemicals Codex, as well as those in the Code (as applicable). Based on those results, the enzyme met all relevant specifications.

[2] International Union of Biochemistry and Molecular Biology.
[3] Chemical Abstracts Service

[4] Information on EC 3.2.1.3 - glucan 1,4-alpha-glucosidase - BRENDA Enzyme Database (brenda-enzymes.org)

[5] For the functional use 'enzyme preparation', the JECFA database can be searched for individual monographs: http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/

Table 2 Analysis of manufacturer's glucoamylase liquid enzyme concentrated preparation compared to JECFA, Food Chemicals Codex, and Code specifications for enzymes.

Test parameters	**Test results**	**Specifications**		
		JECFA	**FCC[1]**	**The Code S3—4**
Lead (mg/kg)	ND	≤5	≤5	≤2
Arsenic (mg/kg)	ND	-	-	≤1
Cadmium (mg/kg)	ND	-	-	≤1
Mercury (mg/kg)	ND	-	-	≤1
Coliforms (cfu[2]/g)	<4	≤30	≤30	
Salmonella (/25 g)	Not detected	Absent	Negative	
E. coli (/25 g)	Not detected	Absent	-	
Antimicrobial activity	Not detected	Absent	-	

[1]Food Chemical Codex, [2]colony forming units

The specification for the enzyme preparation used by the manufacturer (as provided in section 2.5 of the application) includes a test for the absence of the production strain. 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. Total organic solids (TOS) encompasses the enzyme component and other organic material originating from the production organism and the manufacturing process, while excluding intentionally added formulation ingredients.

2.2 Manufacturing process

The glucoamylase is produced by submerged fed-batch pure culture fermentation of the genetically modified strain of *A. niger* followed by a purification process, a formulation process and finally quality control of the finished product. To achieve the enzymatic, physical, and microbial stabilization, sucrose/glucose is added to the enzyme concentrate. The enzyme preparation is manufactured in accordance with current Good Manufacturing Practices. The quality management system used in the manufacturing process complies with ISO 9001:2015.

Full details on the raw materials used for the production were available for this assessment. This information is CCI and cannot be disclosed in this report. After an assessment of all the information (including confidential information), FSANZ agreed that the manufacture of glucoamylase is monitored and controlled adequately to ensure the finished preparation complies with specifications and is suitable as a processing aid in food applications.

2.3 Technological function and justification

Technological function and stability of the glucoamylase enzyme from *A. niger* with different

donor organisms have already been assessed by FSANZ under applications A1248 and A1252. Glucoamylase is intended for use as a processing aid in brewing, distilled alcohol production, starch processing for glucose syrup production and other starch hydrolysates, and in baking.

Glucoamylase converts starch by removing D-glucose units in a stepwise manner from the non-reducing end of the substrate molecule to produce glucose for further processing of food products (refer to section 2.1).

Glucoamylase is principally responsible for the hydrolysis of starch to produce several products such as beer, alcohol, syrups, bread, and other cereal based products (Kumar and Satyanarayana 2009; Marin-Navarro and Polaina 2011).

- In brewing, glucoamylase is used to convert the complex carbohydrates in grains into fermentable sugars, enhancing the alcohol content and ensuring a consistent fermentation process (Guerra *et al*. 2009).
- In the production of spirits, glucoamylase helps in breaking down starches to maximise sugar availability for fermentation (Rasaq *et al.* 2023).
- In baking the enzyme is added to dough to increase the amount of fermentable sugars, which yeast can then convert into carbon dioxide and alcohol, improving the rise and texture of the bread (Guénaëlle Diler *et al.* 2021)
- Glucoamylase is used in the production of natural flavours and syrups, converting starches into sugars which are then used to create sweet flavour profiles (Farooq *et al.* 2020).

The technological purpose as stated by the applicant of glucoamylase in brewing processes, distilled alcohol processes and starch processing for glucose syrups production and other starch hydrolysates, and baking processes is consistent with the typical function of glucoamylase and is supported by scientific literature.

The applicant provided information on the physical and chemical properties of their enzyme preparation. The enzyme is heat-denatured at a temperature greater than 75°C. Therefore, the enzyme is inactivated in final products, and would have no technological effect in the final food.

2.4 Potential presence of allergens

The applicant has provided information as CCI that a raw material that is an allergen (that is required to be declared under the Code) is used during the fermentation process to produce the enzyme. However, analytical testing demonstrates that any presence in the ultra-filtered enzyme concentrate is below the limit of detection for that allergen.[6]

2.5 Food Technology conclusion

FSANZ concludes that the use of this glucoamylase as a processing aid for use as a processing aid in brewing, distilled alcohol production, starch processing for glucose syrup production, other starch hydrolysates, and in baking is consistent with its typical function of hydrolysis with a release of glucose as already assessed by FSANZ under applications A1248 and A1252.

Glucoamylase performs its technological purpose during the production of foods, after which

[6] The applicant has provided the analytical results as confidential commercial information.

it is inactivated, therefore not performing a technological purpose in the final food, and 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

The objectives of this safety assessment are to evaluate any potential public health and safety concerns that may arise from the use of this enzyme, produced by this microorganism, as a processing aid.

Some information relevant to this section is CCI, so full details cannot be provided in this public report.

3.1 History of use of the organisms

3.1.1 Host organism

A. niger is a common, filamentous fungus that is ubiquitous in the environment. In nature it can be found in soil and litter, in compost and on decaying plant material (Schuster *et al.* 2002). *A. niger* has a long history of safe use in industrial-scale enzyme and organic acid production (Cairns *et al.* 2018). This history dates back over 100 years to the discovery of organism's superior properties to produce citric acid (Currie 1917). Citric acid, the primary acidulant in the food and beverage industry, is today mostly produced by microbial fermentation using *A. niger* (Show *et al.* 2015).

FSANZ has previously assessed the safety of *A. niger* as the production organism for several food processing aids. Glucoamylase produced by *A. niger* has previously been approved by FSANZ including with a production organism of the same BO-1 strain lineage as the present application (A1168, A1184, A1248 and A1252). Within the Code, Schedule 18 to Standard 1.3.3 currently also permits the following enzymes derived from this same strain lineage: α-Amylase (A1185), Cellulase (A1271), and Phospholipase A1 (A1221).

A. niger has been reported as a potentially opportunistic pathogen of immunocompromised individuals (Atchade *et al.* 2017; Person *et al.* 2010). However, *A. niger* is regarded as a non-pathogenic fungus to which humans are frequently exposed without disease becoming apparent (Schuster *et al.* 2002). Some strains of *A*. *niger* are capable of producing toxins like ochratoxins and fumonisins, which are harmful to human health (Frisvad *et al.* 2011). The applicant provided CCI information that confirmed the species identification of their *A. niger* production strain and that it belongs to a strain lineage which is non-pathogenic and absent of the mycotoxin and antimicrobial compounds tested for. The applicant demonstrated suitable microbiological controls through production and the absence of the production organism within the final enzyme product. Overall, no public health and safety concerns were identified.

3.1.2 Gene donor organism

The *A. niger* production strain is genetically modified with the glucoamylase gene from *G. sepiarium*. The donor organism's identity was confirmed using information provided by the applicant, which is CCI.

3.2 Characterisation of the genetic modification

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

Multiple copies of an expression cassette containing a protein engineered variant of the glucoamylase gene from *G. sepiarium* were introduced into the genome of the host *A. niger*. The expression cassette contained the *G. sepiarium* glucoamylase gene under the control of an *A. niger*-derived promoter and a terminator derived from either *A. niger* or *Trichoderma reesei*. Data provided by Novozymes and analysed by FSANZ confirmed the identity of the protein engineered glucoamylase enzyme.

The expression cassettes were transformed into protoplasts of the host *A. niger* strain, and transformants were selected based on their ability to grow on selective media and high glucoamylase activity. Two marker genes were introduced into the genome during the transformation and used for the selection of transformants during construction of the production strain.

3.2.2 Characterisation of the inserted DNA

Genome sequencing data provided by the applicant confirmed the presence of the inserted DNA at multiple loci in the genome of the production strain. The data also confirmed the absence of antibiotic resistance genes, marker genes and other plasmid-derived DNA in the final production strain.

3.2.3 Stability of the introduced DNA

The assessment confirmed the inserted gene is stably integrated into the genome of the production strain and does not have the ability to replicate autonomously. The inserted gene is therefore considered to be genetically stable.

To provide further evidence of the stability of the inserted glucoamylase gene, the applicant provided phenotypic data from large-scale fermentation of the production strain, which confirmed the glucoamylase gene is expressed stably over multiple generations.

3.3 Safety of the enzyme

3.3.1 History of safe use

The enzyme is in use in several countries where there are no restrictions of the use of enzyme processing aids or where the enzyme is covered by a country positive list or specific approval. Marín-Navarro and Polaina 2011). The Danish Veterinary and Food Administration authorised the use of the enzyme preparation in 2023.

Glucoamylases have been used in commercial food production since the 1960s to hydrolyse starch to fermentable sugars (Godfrey 1983; Janda 1983; Poulson 1983; Reichelt 1983; van Oort 2010). Glucoamylases from various sources are widely authorised internationally, and the Code authorises the use of glucoamylases from five species of production organism.

3.3.2 Bioinformatics concerning potential for toxicity

Protein sequences that contain the word ‘toxin’ in the description field were extracted from UNIPROT (Database date: 2021-02-15). A total of 333068 entries were found. Each of the sequences was placed in its uniquely named Fasta file. The glucoamylase that is the subject of this application was placed in a separate file named glucoamylase.fasta. The sequence alignment program ClustalW 2.0.10 was invoked to align each ‘toxin’ sequence to the glucoamylase enzyme. A summary file containing the length of each sequence and number of identical residues was also created. From this, the identity percentage to the glucoamylase sequence or the compared toxin sequence was calculated, whichever was longest. The largest homology encountered was 17.5%, indicating that the homology to any toxin sequence in this database is random and very low.

3.3.3 Toxicity data

Toxicity studies were conducted using the enzyme concentrate prior to the stabilization and standardisation steps of the enzyme production process. The animal study and the genotoxicity studies were all conducted using the same representative batch of enzyme concentrate, designated PPY75245.

3.3.3.1 Animal study

Thirteen-week oral gavage study of glucoamylase enzyme concentrate in Han Wistar rats (Labcorp 2022). Regulatory status: GLP, conducted in compliance with OECD Guideline 408

The enzyme concentrate was a liquid at room temperature and contained 10.3% w/w TOS. Dose levels were 0, 10%, 33% and 100% of the enzyme concentrate, equivalent to 0, 107.0, 353.2 or 1070.2 mg TOS/kg body weight (bw)/day. The vehicle and control article were reverse osmosis water. Han Wistar rats, 10/sex/group, were acclimatized to standard laboratory environmental conditions for 12 days prior to commencement of dosing and were 41 to 47 days old when dosing began. Rats of the same sex and dose group were group-housed, five per cage.

Rats were dosed once daily at a dose volume of 10 mL/kg bw. Cage-side inspections were made at least twice daily. Detailed examinations were recorded daily during the first week, twice weekly from weeks 2 to 4, and weekly thereafter. These included physical examinations and observations of behaviour and movement in an arena. A functional observational battery of sensory reactions and grip strength was recorded for all rats in Week 12. Bodyweights and food consumption were recorded weekly. Ophthalmic examinations were conducted pre-study and during Week 12. Oestrous activity was assessed by vaginal smears of females for four consecutive days during Week 13.

Prior to scheduled termination in Week 13, rats were anaesthetised, and blood was collected for haematology, blood chemistry and thyroid hormone analysis. While still under anaesthesia, rats were killed by carbon dioxide asphyxiation, and detailed necropsy was performed. Sperm was collected from the left vas deferens of males. Fresh weights of a range of organs were recorded and a comprehensive list of organs and tissues were preserved for microscopic examination.

Analysis for nitrogen concentration of the dose formulations in Weeks 1, 6 and 13 demonstrated correct dose formulations. Except for one male rat in the 33% group that was found dead in Week 6, all rats survived to scheduled termination. The rat was subject to necropsy and microscopic examination of tissues but cause of death was not determined. There were no treatment-related effects on clinical observations, sensory reactivity, grip

strength, bodyweight gain, food consumption, ophthalmology, haematology, blood chemistry, thyroid hormones, oestrus cycles, organ weights, gross necropsy findings, histopathology findings or spermatogenesis in males. It was concluded that the No Observed Adverse Effect Level (NOAEL) was 1070.2 mg TOS/kg bw/day, the highest dose tested.

3.4.3.2 Genotoxicity studies

Bacterial reverse mutation test of glucoamylase concentrate (Labcorp 2021). Regulatory status: GLP, conducted in compliance with OECD Test Guideline 471

The test article for this test was the same batch of enzyme concentrate, PPY75245, as that used for the 13-week study in rats. The bacterial test strains used were *Salmonella enterocolitica* var. Typhimurium strains TA1535, TA1537, TA98 and TA100, and *Escherichia coli* strain WP2 *uvrA*. The tests were performed using the treat-and-wash method, in the presence and absence of S9 mix for metabolic activation. Reverse osmosis was used as the vehicle and negative control article. Appropriate positive control articles were used for positive control tests run concurrently.

Concentrations of glucoamylase up to 5000 µg TOS/mL were tested. Two independent tests were conducted in succession. In the absence of any signs of toxicity or mutagenesis in the first test, the only modification made between the first and second test was an increase in the concentration of S9 mix from 10% to 20%.

Aliquots of test cultures were distributed into conical tubes. Two sets of tubes were prepared so that each test substance concentration was tested with and without S9 mix. S9 mix phosphate buffer were added as appropriate and the tubes treated with the enzyme concentrate, vehicle or positive control solution were added. Cultures were incubated for 90 minutes at 34 to 39°C with shaking. After incubation, the bacteria were washed twice by suspension in wash solution and centrifugation, and then mixed thoroughly to agar containing histidine, biotin, and tryptophan. The mixtures were overlaid onto labelled Petri dishes containing minimal agar.

Petri dishes were prepared for each test substance concentration and for the vehicle and positive controls. All plates were incubated at 34 to 39°C for 72 hours. After incubation, the appearance of the background bacterial lawn was examined and revertant colonies were counted.

No precipitation of test article occurred at any concentration in either test, or no signs of toxicity towards the tester strains were observed in either test. No evidence of mutagenic activity was seen at any concentration of glucoamylase in either test. Mean counts of revertant colonies in the negative control assays were within the historical control range of the testing laboratory. Expected increases in reverse mutation in the presence of positive control articles confirmed the validity of the test.

It was concluded that the enzyme concentrate showed no evidence of mutagenic activity under the test conditions.

In Vitro *micronucleus test of glucoamylase concentrate in human lymphocytes (Covance 2021). Regulatory status: GLP, conducted in compliance with OECD test guideline 487.*

The test article for this test was the same batch of enzyme concentrate, PPY75245, as that used for the 13-week study in rats and the bacterial reverse mutation assay. The lymphocytes were obtained from two healthy non-smoking donors. Two tests, a preliminary toxicity test and a main test for induction of micronuclei, were conducted. Lymphocytes were exposed to the test article for three hours both in the presence and absence of S9 mix, and for 20 hours in the absence of S9 mix, at concentrations up to 5000 µg TOS/mL. The

vehicle/negative control was reverse osmosis water, and appropriate positive control assays were conducted concurrently.

The enzyme concentrate did not cause any statistically significant increase in micronuclei under any of the test conditions used, when compared to the vehicle controls. The mean micronucleus frequencies in negative control and enzyme-treated cultures were all within the historical control range for the test laboratory. The positive control articles induced statistically significant increases in the number of binucleate cells containing micronuclei, confirming the validity of the assay. It was concluded that the enzyme concentrate did not cause an increase in the induction of micronuclei under the test conditions.

3.3.4 Potential for allergenicity

A sequence homology assessment of the glucoamylase enzyme to known allergens was conducted by comparing the amino acid sequence of the glucoamylase to allergens from the FARRP allergen protein database (http://www.allergenonline.org).

Homology was identified to one known respiratory allergen above the threshold of 35 % across an 80 amino acid window. The assessment identified 81.25 % and 81.01 % (without and with scaling) to Sch c 1, a glucoamylase originating from *Schizophyllum commune*. Sch c 1 has been found to act as a respiratory allergen in occupational settings but is not known to be a food allergen.

The great majority of adults affected by occupational asthma can ingest the respiratory allergen to which they are allergic without acquiring clinical symptoms of food allergy (Brisman, 2002; Poulsen, 2004; Armentia *et al.*, 2009). It is therefore considered to be unlikely that oral exposure to the glucoamylase that is the subject of this application would pose any allergenic concern.

3.3.5 Safety assessments by other agencies

No safety assessments by other agencies are available. FSANZ notes that Denmark approved this glucoamylase in 2023.

3.3.6 Toxicology conclusions

The results of bioinformatics searches showed no homology with known toxins or venoms. Similarly, no significant homology with known food allergens was identified in recent bioinformatics searches of the AllergenOnline database. A thirteen-week oral gavage study of the enzyme in rats established a NOAEL of 1070.2 mg TOS/kg bw/day, the highest dose tested. There was no evidence of genotoxicity of the enzyme in a bacterial reverse mutation assay or a micronucleus assay in human lymphocytes.

3.4 Dietary Exposure

The objective of the dietary exposure assessment was to review the budget method calculation presented by the applicant as a ‘worst-case scenario’ approach to estimating levels of dietary exposure if all the TOS from the Glucoamylase enzyme preparation remained in the food.

The budget method is a valid screening tool for estimating the theoretical maximum daily intake (TMDI) of a food additive (Douglass *et al.* 1997). The calculation is based on physiological food and liquid requirements, the food additive concentration in foods and beverages, and the proportion of foods and beverages that may contain the food additive.

The TMDI can then be compared to an Acceptable Daily Intake (ADI) or a NOAEL to estimate a margin of exposure (MOE) for risk characterisation purposes. Whilst the budget method was originally developed for use in assessing food additives, it is also appropriate to use for estimating the TMDI for processing aids (FAO/WHO 2020). International regulatory bodies and the FAO use the method/WHO Joint Expert Committee on Food Additives (JECFA) (FAO/WHO 2021) for dietary exposure assessments for processing aids.

In their budget method calculation, the applicant made the following assumptions:

- the maximum physiological requirement for solid food (including milk) is 25 g/kg body weight/day
- Fifty percent of solid food is processed
- Processed solid food contains 25% starch (or starch-derived) dry matter
- all solid foods contain the highest use level of 590 mg TOS/kg of enzyme preparation in the raw material (flour)
- the maximum physiological requirement for liquid is 100 mL/kg body weight/day (the standard level used in a budget method calculation for non-milk beverages)
- Twenty-five percent of non-milk beverages are processed
- Processed non-milk beverages contain 12% starch (or starch-derived) dry matter
- all non-milk beverages contain the highest use level of 821 mg TOS/kg in the raw material (starch (or starch-derived) dry matter)
- the densities of non-milk beverages are ~1
- all the TOS from the enzyme preparation remains in the final food.

Based on these assumptions, the applicant calculated the TMDI of the TOS from the enzyme preparation to be 4.30 mg TOS/kg bw/day.

As assumptions made by the applicant differ from those that FSANZ would have made in applying the budget method, FSANZ independently calculated the TMDI using the following assumptions that are conservative and reflective of a first tier in estimating dietary exposure:

The maximum physiological requirement for solid food (including milk) is 50 g/kg body weight/day (the standard level used in a budget method calculation where there is potential for the enzyme preparation to be in baby foods or general-purpose foods that would be consumed by infants).

FSANZ would assume 12.5% of solid foods contain the enzyme based on commonly used default proportions noted in the FAO/WHO Environmental Health Criteria (EHC) 240 Chapter 6 on dietary exposure assessment (FAO/WHO 2009). However, the applicant has assumed a higher proportion of 50% based on the nature and extent of use of the enzyme and therefore FSANZ has also used this proportion for solid foods as a worst-case scenario.

All other inputs and assumptions used by FSANZ remained as per those used by the applicant. The TMDI of the TOS from the enzyme preparation based on FSANZ's calculations for solid food and non-milk beverages is 6.15 mg TOS/kg bw/day.

Both the FSANZ and applicant's estimates of the TMDI will be overestimates of the dietary exposure given the conservatisms in the budget method. This includes that it was assumed that all the TOS from the enzyme preparation remains in the final foods and beverages. Whereas the applicant has stated that the enzyme is denatured by heat during processing or removed by down-stream processes and does not have a function in the final food to which the ingredient is added.

4 Conclusion

FSANZ concludes that the proposed use of glucoamylase from GM *A. niger* as a processing aid in baking processes, brewing processes, and starch processing to produce starch hydrolysates, including glucose syrups is consistent with its known technological function of hydrolysis with a release of glucose. Analysis of the evidence provides adequate assurance that the use of this enzyme, in the quantity and form proposed to be used at levels consistent with GMP, is technologically justified. The enzyme meets international purity specifications.

Glucoamylase performs its technological purpose during the production of food and is not performing a technological purpose in the final food. It is therefore appropriately categorised as a processing aid as defined in the Code.

No public health and safety concerns were identified in the assessment of glucoamylase from GM *A. niger* under the proposed use conditions. The *A. niger* host is neither pathogenic nor toxigenic. Analysis of the modified production strain confirmed the presence and stability of the inserted DNA.

Bioinformatic analysis indicated that the enzyme shows no significant homology with any known toxins. Glucoamylase from GM *A. niger* (gene donor*: G. sepiarium*) was not genotoxic in vitro. A NOAEL of 1070.2 mg TOS/kg bw/day was identified in a 13-week oral toxicity study in rats. The TMDI was calculated by FSANZ to be 6.15 mg TOS/kg bw/day. A comparison of the NOAEL and the TMDI results in a large MOE of approximately 200.

Based on the reviewed data it is concluded that, in the absence of any identifiable hazard, an ADI ‘not specified’ is appropriate.

5 References

Atchade E, Jean-Baptiste S, Houzé S, Chabut C, Massias L, Castier Y, Brugière O, Mal H, Montravers P (2017) Fatal invasive aspergillosis caused by *Aspergillus niger* after bilateral lung transplantation. Medical mycology case reports 17:4-7

Cairns TC, Nai C, and Meyer V (2018) How a fungus shapes biotechnology: 100 years of *Aspergillus niger* research. *Fungal Biol Biotechnol* **5**, 13.

Currie JN (1917) The citric acid fermentation of *Aspergillus niger*. J Biol Chem. 31:15–37.

Douglass JS, Barraj LM, Tennant DR, Long WR and Chaisson CF (1997) 'Evaluation of the Budget Method for screening food additive intakes, Food Additives and Contaminants' 14:791-802.

FAO/WHO (2009) 'Environmental Health Criteria 240. Principles and Methods for the Risk Assessment of Chemicals in Food' Chapter 6 – Dietary exposure assessment of chemicals in food, WHO, Geneva.

FAO/WHO (2020) Environmental Health Criteria 240. Principles and Methods for the Risk Assessment of Chemicals in Food. Chapter 6: Dietary exposure assessment of chemicals in food. Second Edition 2020. WHO, Geneva. https://www.who.int/docs/default-source/food-safety/publications/chapter6-dietary-exposure.pdf?sfvrsn=26d37b15_6

FAO/WHO (2021) Evaluation of certain food additives: eighty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No. 1027.

Farooq, M.A., Ali, S., Hassan, A. et al. Biosynthesis and industrial applications of α-amylase: a review. Arch Microbiol 203, 1281–1292 (2021). https://doi.org/10.1007/s00203-020-02128-y

Frisvad JC, Larsen TO, Thrane U, Meijer M, Varga J, Samson RA, Nielsen KF (2011) Fumonisin and ochratoxin production in industrial *Aspergillus niger* strains. PLoS One 6(8): e23496

Guénaëlle Diler, Cécile Rannou, Claire Guyon, Carole Prost, Alain Le-Bail (2021) *Use of amyloglucosidase in a soft wheat dough: Impact of process and formulation on glucose production*, Applied Food Research,Volume 1, Issue 2,100007,ISSN 2772-5022,https://doi.org/10.1016/j.afres.2021.100007.

Jafari-Aghdam, J.; Khajeh, K.; Ranjbar, B.; Nemat-Gorgani, M. (2005) Deglycosylation of glucoamylase from Aspergillus niger: effects on structure, activity, and stability (2005), Biochim. Biophys. Acta, 1750, 61.

Kumar, P., & Satyanarayana, T. (2009). Microbial glucoamylases: characteristics and applications. Critical Reviews in Biotechnology, 29(3), 225–255. https://doi.org/10.1080/07388550903136076

Marín-Navarro J, Polaina J. Glucoamylases: structural and biotechnological aspects. Appl Microbiol Biotechnol. 2011 Mar;89(5):1267-73.

N.P. Guerra, A. Torrado-Agrasar, C. López-Macías, E. Martínez-Carballo, S. García-Falcón, J. Simal-Gándara, L.M. Pastrana-Castro (2009) *Use of Amylolytic Enzymes in Brewing*, Editor(s): Victor R. Preedy, Beer in Health and Disease Prevention, Academic Press,2009, Pages 113-126,

Person AK, Chudgar SM, Norton BL, Tong BC, Stout, JE (2010) *Aspergillus niger*: an unusual cause of invasive pulmonary aspergillosis. Journal of Medical Microbiology

59(7):834-838

Rasaq S. Abolore, Bahiru Tsegaye, Swarna Jaiswal, Amit K. Jaiswal (2023) *Chapter 1 - An overview of industrial enzymes in beverage production and processing,* Editor(s): Mohammed Kuddus, Mohammad Hossain, Value-Addition in Beverages through Enzyme Technology,Academic Press,2023,Pages 1-26,

Schuster E, Dunn-Coleman N, Frisvad JC, van Dijck PWM (2002) On the safety of *Aspergillus niger* - a review. Appl Microbiol Biotechnol 59:426-435

Show PL, Oladele KO, Siew QY, Aziz Zakry FA, Lan JC, Ling TC (2015) Overview of citric acid production from *Aspergillus niger*. Frontiers in Life Science 8(3):271-283