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Supporting document 1

Risk and technical assessment – Application A1252

Glucoamylase from GM *Aspergillus niger* (gene donor: *Penicillium oxalicum*) as a processing aid

Executive summary

Novozymes Australia Pty Ltd (Novozymes) submitted an application to permit the use of a protein engineered variant of glucoamylase (EC 3.2.1.3) from genetically modified (GM) *Aspergillus niger* (*A. niger*), containing the glucoamylase gene from *Penicillium oxalicum* (*P. oxalicum*). The glucoamylase is proposed for use as a processing aid in baking processes, brewing processes and starch processing for the production of starch hydrolysates, including glucose syrups, in accordance with Good Manufacturing Practice (GMP) conditions.

FSANZ has undertaken an assessment and concludes that the proposed use of glucoamylase as a processing aid in baking processes, brewing processes and starch processing for the production of starch hydrolysates, including glucose syrups is consistent with its known technological function of hydrolysing carbohydrates, with the 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.

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.

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.

Glucoamylase does not show any appreciable sequence homology with known toxins. The enzyme was not genotoxic *in vitro*, and no treatment-related adverse effects were observed in a 13-week oral toxicity study in rats. The no observed adverse effect level (NOAEL) was the highest dose tested, 1360 mg total organic solids (TOS)/kg bw/day. No significant homology to known food allergens was identified. Some homology to a respiratory allergen from *S. commune* (splitgill mushroom) was identified, however respiratory allergens are not usually food allergens and *S. commune* is consumed as a food overseas without reports of food allergy. Based on the available evidence the enzyme is unlikely to pose a food allergenicity concern.

The theoretical maximum daily intake (TMDI) for solid food and non-milk beverages was calculated as 8.97 mg TOS/kg bw/day. Comparison of the NOAEL and the TMDI results in a margin of exposure (MOE) of around 150.

In the absence of any identifiable hazard an acceptable daily intake (ADI) 'not specified' is appropriate for glucoamylase from *P. oxlicum*, expressed in GM *A. niger*.

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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) as a processing aid in baking processes, brewing processes and starch processing for the production of starch hydrolysates, including glucose syrups. This enzyme is sourced from a genetically modified (GM) strain of *Aspergillus niger* containing a protein engineered variant of the glucoamylase gene from *Penicillium oxalicum*. The enzyme is prepared as a liquid or granulated preparation under the commercial name Attenuzyme Fast.

Schedule 18 of the Australia New Zealand Food Standards Code (the Code) includes permission for two other glucoamylase enzymes (not protein engineered) produced by *Aspergillus niger* containing the glucoamylase gene from *Talaromyces emersonii* or *Trametes cingulata*. Therefore, this protein engineered variant of the glucoamylase enzyme produced by GM *A. niger* containing the glucoamylase gene from *P. oxalicum* needs pre-market assessment before permission can be given for its use as a processing aid. If permitted, the enzyme will provide an option for baking processes, brewing processes and starch processing for the production of starch hydrolysates, including glucose syrups.

1.1 Objectives of the assessment

The objectives of this risk and technical assessment were to:

- determine whether the proposed purpose is clearly stated and that the enzyme achieves its technological function 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, produced by a GM microorganism, as a processing aid, specifically by considering the:
 - history of use of the gene donor and production microorganisms
 - characterisation of the genetic modification(s), and
 - safety of the enzyme.

2 Food technology assessment

2.1 Characterisation of the enzyme

2.1.1 Identity and properties of the enzyme

The production microorganism of the enzyme is a GM strain of *A. niger*. The donor microorganism of the glucoamylase gene is *P. oxalicum* (further details contained in Section 3). The applicant provided relevant information regarding the identity of the enzyme, and this has been verified using the IUBMB enzyme nomenclature database (IUBMB, 2018). Details of the identity of the enzyme are provided below.

Accepted IUBMB¹ name: Glucan 1,4- α -glucosidase

¹ International Union of Biochemistry and Molecular Biology

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 ² :	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

Details on the raw materials and ingredients used in the production of the glucoamylase enzyme preparation were provided in the application or as Commercial in Confidence (CCI).

The enzyme is produced by submerged fermentation of the *A. niger*, carrying the glucoamylase gene from *P. oxalicum*. The fermentation processes are consistent with the scientific literature and references provided by Novozymes (Aunstrup 1979). All preparations are completed aseptically in accordance with Good Manufacturing Practices (GMP). Novozymes has provided certificates for compliance with ISO 9001:2015.

The fermentation process starts with the preparation of the medium including carbon, nitrogen, vitamins and minerals. The pH is adjusted and additional processing aids are used as antifoaming agents. This is then followed by inoculum, seed fermentation, main fermentation and the recovery stage to separate the enzyme from the biomass and to purify, concentrate and stabilize it. Ultrafiltration and/or evaporation are used for additional concentration and purification. The final enzyme preparation will depend on the intended use, for example, remain as a single enzyme preparation or be blended with other enzymes to form a granulate.

Appropriate quality control processes are in place to prevent any contamination during the fermentation process. The application states that all raw materials used in the fermentation and recovery processes are standard ingredients of food grade quality that meet predefined quality standards. The raw materials conform to either specifications set out in the Food Chemical Codex, 12th edition, 2020 or regulations applying in the European Union. The ingredient list of raw materials has been sighted and although the information has been provided as CCI, the list has been confirmed as being permitted by the Code.

The applicant has advised that wheat flour is used for the carrier and has confirmed that wheat protein is present in the final enzyme preparation³. This only applies to the granulated preparation which is used in baking processes. The Product Data Sheet lists the presence of cereals containing gluten (i.e. wheat, rye, barley, oats spelt, kamut). It is worth noting that the enzyme will be used in bakery products largely containing wheat. Section 3.3.4 provides

² Chemical Abstracts Service

more information on the allergenicity associated with the enzyme and is further discussed in Section 2.2.3 of the Call for Submissions document.

2.2.2 Specifications

There are international 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, 2017) and the Food Chemicals Codex (FCC) (USPC, 2018). These specifications are included in the primary sources listed in section S3—2 of Schedule 3 of the Code and enzymes used as a processing aid must meet either of these specifications. Schedule 3 of the Code also includes specifications for arsenic and metals (section S3—4) if they are not already detailed within specifications in sections S3—2 or S3—3.

Table 1 provides a comparison of the analysis of three batches of the glucoamylase enzyme with international specifications established by JECFA and Food Chemicals Codex, as well as those in the Code (as applicable). Based on these results, the enzyme meets all relevant specifications. Certificates of analysis have been provided which confirm the results below.

Table 1 Analysis of enzyme glucoamylase compared to JECFA, Food Chemicals Codex, and Code specifications for enzymes (three batches)

Analysis	Results from Applicant	JECFA	Food Chemicals Codex	Australia New Zealand Food Standards Code (section S3-4)
Lead (mg/kg)	ND	≤ 5	≤ 5	≤2
Arsenic (mg/kg)	ND	-	-	≤1
Cadmium (mg/kg)	ND	< 0.5	-	≤1
Mercury (mg/kg)	ND	< 0.5	-	≤1
Coliforms (cfu/g)	<10	≤30	≤30	-
<i>Salmonella</i> (in 25 g)	ND	Absent	Negative	-
<i>E. coli</i> (in 25 g)	ND	Absent	-	-
Antimicrobial activity	ND	Absent	-	-

2.3 Technological purpose of the enzyme

The enzyme is intended to be used as a processing aid in baking, brewing and starch processing for the production of starch hydrolysates, including glucose syrups. The purpose of glucoamylase is hydrolysis of terminal (1->4)-linked alpha-D-glucose residues from non-reducing ends of the chains with release of beta-D-glucose (BRENDA:EC3.2.1.3, 2022) - see Figure 1.

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). Table 2 includes a summary of the physical and chemical properties of the enzyme.

Figure 1. Hydrolysis reaction catalysed by glucoamylase

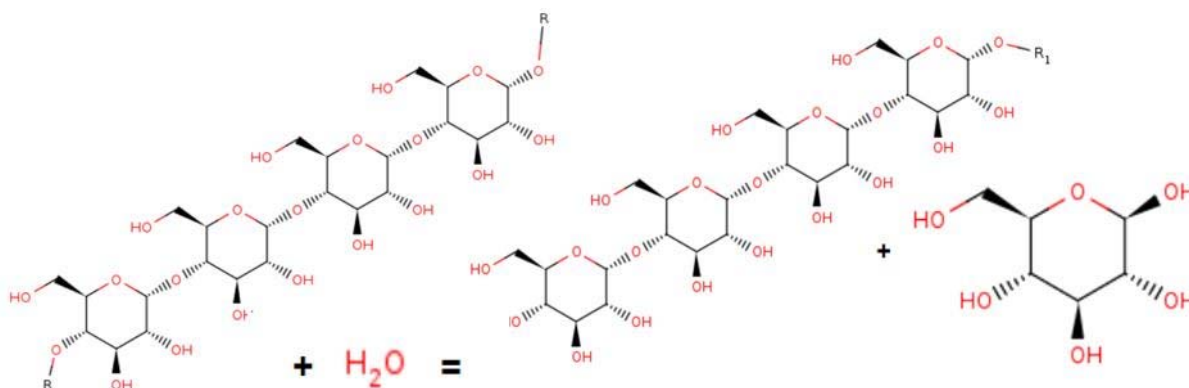
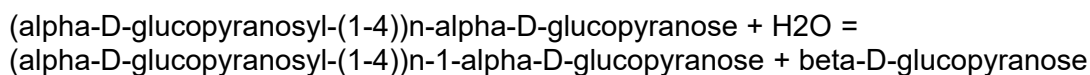


Table 2 Glucoamylase enzyme physical/chemical properties

<i>Physical/chemical properties</i>	<i>Liquid preparation</i>	<i>Granulated preparation</i>
<i>Enzyme activity</i>	420 AGU/g (Amyloglucosidase unit)	2500 AGU/g
<i>Appearance</i>	Light to dark brown	Off-white to light brown
<i>Temperature optimum</i>	60-75°C at pH 4	Not provided
<i>Temperature stability/storage</i>	0-10°C	0-25°C
<i>pH optimum</i>	3.5-4.5 at 37°C	Not provided
<i>pH stability</i>	5	Not provided

Following the hydrolysis reaction, residual proteins will be removed through washing and/or filtration. Using the enzyme in bakery products at high oven temperatures will result in it being inactivated. Similarly, brewing and starch hydrolysis will also reach these elevated temperatures and inactivate the enzyme. Information provided by Novozymes states that the enzyme is inactivated at 80°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 where it is present in negligible amounts in the final food and have no ongoing technical function.

The stability of glucoamylase varies between the liquid and granulated product. A product specification sheet for both the liquid and granulated products was provided.

2.4 Technological justification of the enzyme

Novozymes claim that this glucoamylase is more efficient than currently permitted glucoamylases. Novozymes has highlighted the following potential functions provided by the enzyme.

In baking processes, the glucoamylase degrades starch and dextrins into glucose that can be fermented by yeast.

In brewing processes, the glucoamylase degrades starch into fermentable sugars, used to create alcohol.

- more uniform and predictable production process and brewing yield including the possibility to control the desired level of fermentable sugars.

In starch hydrolysis and glucose syrup production, the glucoamylase degrades polysaccharides into glucose for further processing.

- efficient degradation of dextrins and production of glucose
- reduced risk of microbial contamination because of use at high temperatures
- stable process allowing for variations in pH and temp
- increased purity of the product compared to acid-acid process

Novozymes's glucoamylase enables the effective hydrolysis of glucose from starches. Baking, brewing and starch hydrolysis may use different starches (e.g. flour or malt) in their production, with glucoamylase enabling removal of glucose during each process.

In baking, the availability of the glucose assists with the yeast functionality by providing an energy source for yeast to ferment. Glucoamylase is also used to improve crust colour and enhance the quality of high fibre products (Raveendran et al. 2018). In brewing, the enzyme can be added during fermentation to metabolise dextrins (residual carbohydrates) turning 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.

A study published by the European Food Safety Authority (EFSA) on the safety of the food enzyme, glucoamylase from a GM *A. niger*, considered the same functions as the scope of the current application (EFSA 2018) and found the enzyme to be safe for the intended uses.

Glucoamylase enzymes from multiple sources have already been approved for use in many countries. An independent safety assessment completed by the Danish Veterinary and Food Administration was provided by Novozymes and reviewed as part of this assessment.

2.5 Food technology 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 for the production of starch hydrolysates, including glucose syrups, is consistent with its known technological function of hydrolysing carbohydrates to 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.

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

3.1 History of use

3.1.1 Host organism

A. niger is widely used as a production organism and host for the manufacture of food ingredients and enzymes. *A. niger* is recognised as neither pathogenic nor toxigenic. FSANZ has previously assessed the safety of host organisms from the *A. niger* BO-1 strain lineage most recently in applications A1221 and A1248. The identity of the host organism was determined using standard molecular techniques (Houbraken et al. 2020).

3.1.2 Gene donor organism

The donor of the glucoamylase gene is identified as *P. oxalicum*. The species name *P. oxalicum* is accepted in the genus *Penicillium* (Houbraken et al. 2020).

3.2 Characterisation of the genetic modification(s)

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

An expression cassette containing the glucoamylase gene was introduced into the *A. niger* host strain's genome, producing the production strain. The glucoamylase gene is derived from *P. oxalicum* and is under the control of the native promoter and terminator from *A. niger*. A selectable marker gene enabling the selection of positive transformants by growth on media supplemented with acetamide was used. Data provided by Novozymes and analysed by FSANZ confirmed the expected glucoamylase amino acid sequence. The glucoamylase enzyme has been protein engineered and differs from the wild type glucoamylase enzyme by a single amino acid.

A vector containing the glucoamylase expression cassette was used to transform the host strain. The expression cassette was integrated at specific integration sites in the host's genome. The final production strain was selected based by growth on medium containing acetamide and high glucoamylase activity.

3.2.2 Characterisation of inserted DNA

Data provided by Novozymes and analysed by FSANZ confirmed the presence of the inserted DNA in the production strain. The applicant also provided Southern blot analysis which confirmed the absence of antibiotic resistance genes in the production strain.

3.2.3 Genetic stability of the inserted gene

The assessment confirmed the inserted gene is 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 introduced glucoamylase gene, the

applicant provided phenotypic data from large-scale fermentation of the production strain. These data confirmed that the glucoamylase gene is expressed over multiple generations and is stable.

3.3 Safety of glucoamylase

3.3.1 History of safe use of the enzyme

Glucoamylases have a long history of use in industrial food applications, with major application in the starch, distilling, brewing and baking industry (Godfrey 1983; Janda 1983; Poulson 1983; Reichelt 1983; van Oort 2010). Glucoamylases from a variety of sources are authorised in a range of countries including Australia and New Zealand, Brazil, Canada, China, Denmark, France, Japan and Mexico.

The applicant has indicated that their glucoamylase enzyme preparation is currently in use in a range of countries, and has been authorised for use in Denmark since 2019.

3.3.2 Bioinformatics concerning potential for toxicity

The applicant performed an assessment of amino acid sequence homology of the glucoamylase enzyme to known toxins included in the [UniProt database](#). No significant homologies were identified.

3.3.3 Toxicology data

The test item used in the following studies was glucoamylase, batch PPY34422, an enzyme concentrate representative of the glucoamylase subject to this application before the addition of other components of the commercial food enzyme preparation.

3.3.3.1 Animal studies

13-week oral toxicity study in rats (Huntingdon Life Sciences 2013) Regulatory status: GLP; conducted in accordance with OECD TG 408 (1998)

Glucoamylase was administered to Sprague-Dawley [CrI:CD(SD)] rats (10/sex/group) at doses of 0, 136, 449 or 1360 mg total organic solids (TOS)/kg bw/day for 13 weeks. Water was used as the vehicle control. Clinical condition was monitored daily. Body weight, food consumption and detailed clinical examinations for signs of toxicity were recorded weekly. Sensory activity, grip strength and motor activity assessments were performed on all animals during Week 12 of treatment. Ophthalmic examination was conducted on all animals prior to study initiation and on control and high-dose animals in Week 12. At the end of the study blood samples were collected for haematology and clinical chemistry analysis. Gross pathology and measurement of organ weights was conducted on all animals at study termination, and organs and tissues from the control and high-dose group animals underwent histopathological examination.

All animals survived to the end of the study and no treatment-related clinical signs were observed. No treatment-related effects were observed on feed consumption, body weight and body weight gain, sensory reactivity, grip strength, motor activity, ophthalmology, haematology or clinical chemistry parameters. Organ weights were not affected by treatment and no treatment-related macroscopic or histopathologic changes were observed.

It was concluded that the no observed adverse effect level (NOAEL) in this study was 1360

mg TOS/kg bw/day, the highest dose tested.

3.3.3.2 Genotoxicity studies

Bacterial reverse mutation test (Novozymes 2013) Regulatory status: GLP; conducted in accordance with OECD TG 471

Glucoamylase was tested for mutagenicity in the *Salmonella enterica* ser. Typhimurium strains TA1535, TA100, TA1537, TA98 and *Escherichia coli* WP2uvrA (pKM101). The treat and plate method was used because the enzyme preparation contained histidine and tryptophan which could potentially lead to false positive results. The assays were performed in triplicate in the presence and absence of metabolic activation (S9 mix). Results were confirmed by conducting each assay for a second time. Test item concentrations ranged from 156 – 5000 µg/mL. The vehicle control was water. Appropriate positive control articles were used to confirm the validity of the assay.

No increases in the number of revertant colonies that met the criteria for a positive or equivocal response were observed following treatment with the enzyme. Slightly increased numbers of revertant colonies compared with controls were observed in TA100 in the absence of S9 in experiment 1, however a dose response was not observed, and similar changes were not observed in experiment 2. Experiment 2 also included an assessment of cell viability in TA100 in the absence of S9 which demonstrated a growth stimulating effect of the test item, which was likely to be the cause of the increased colony numbers seen in experiment 1. All positive control chemicals induced significant increases in revertant colony numbers, confirming the validity of the test system.

It was concluded that glucoamylase PPY34422 was not mutagenic under the conditions of this study.

In vitro mammalian cell micronucleus test (Covance 2013) Regulatory status: GLP; conducted in accordance with OECD TG 487

Glucoamylase was tested for its ability to induce micronuclei in human lymphocytes. Cells were either exposed to the test substance in the presence or absence of S9 for 3 hours (short-term), or exposed to the test substance for 24 hours (long-term) without S9. In the short-term study cells were cultured for a further 21 hours following exposure to the test item in the presence of cytochalasin B, while in the long-term study cells were cultured for an additional 24 hours with cytochalasin B. Glucoamylase concentrations up to 5000 µg/mL were tested based on results of a dose-range finding study. The vehicle control was water. Mitomycin C and vinblastine were used as clastogenic and aneugenic positive controls, respectively, in the absence of S9. Cyclophosphamide was the positive control in the presence of S9. Cultures were performed in duplicate and 1000 binucleated cells from each culture were scored for the presence of micronuclei (2000 per treatment).

Short-term treatment with glucoamylase in the absence of S9 did not result in significant increases in the frequency of micronucleated binucleate (MNBM) cells compared with vehicle controls. Following short-term treatment in the presence of S9, the MNBN cell frequency for all but a single replicate culture at 5000 µg/mL was similar to vehicle controls. However, analysis of additional cells from both of the replicate cultures at this concentration demonstrated an overall response that was not different from the vehicle control, and within the laboratory's historical vehicle control values. As such, this isolated observation was considered spurious. A statistically significant increase in the frequency of MNBN cells was observed at 5000 µg/mL following long-term treatment, but there was no evidence of a dose-

response, the increase was small, and it fell within the historical control range. This slight increase was therefore not considered of biological importance. All positive controls induced significant increases in the proportion of cells with micronuclei, confirming the validity of the test system.

It was concluded that glucoamylase PPY34422 did not induce micronuclei under the conditions of this study.

3.3.4 Potential for allergenicity

The applicant provided details of the following searches for amino acid sequence homology of the glucoamylase enzyme to known allergens, using the [AllergenOnline](#) database:

- 35% identity over 80 amino acids
- 35% identity over 80 amino acids with scaling enabled
- Full length alignment
- 100% identity over 8 contiguous amino acids

The sequence homology assessment found matches with > 35% identity in the first three searches, and matches with 100% identity over 8 amino acids, to Sch c 1, a glucoamylase originating from *Shizophyllum commune*. Sch c 1 has been identified as a respiratory allergen but not as a food allergen. *S. commune* (splitgill mushroom) is eaten in Africa, Asia, the Indian subcontinent, and Central America but is not associated with reports of food allergy.

Respiratory allergens are not usually food allergens, and studies have indicated that individuals with occupational respiratory allergies can ingest the respiratory allergen without developing food allergy (Armentia et al. 2009; Cullinan et al. 1997; Poulsen 2004). No food allergy responses to 19 microbially-derived food enzymes, including a glucoamylase from *A. niger*, were found in a study of 400 individuals with allergy to inhalation, food or other allergens (Bindslev-Jensen et al. 2006).

Based on the available evidence, taken together with the very low levels of glucoamylase expected to be present in the final foods following use, the risk of food allergy from use of the glucoamylase enzyme is likely to be negligible.

3.3.5 Assessments by other regulatory agencies

The enzyme has been approved for use in Denmark, but no safety assessment underlying this approval is publicly available.

3.4 Dietary exposure assessment

The objective of the dietary exposure assessment was to review the budget method calculation presented by the applicant as a 'worse-case scenario' approach to estimating likely levels of dietary exposure, assuming all added glucoamylase enzyme from GM *A. niger* (containing the glucoamylase gene from *P. oxalicum*) 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 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). The method is used by international regulatory bodies and the FAO/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 of solid foods (including milk) is 25 g/kg body weight/day
- the maximum physiological requirement for non-milk beverages is 100 mL/kg body weight/day (the standard level used in a budget method calculation)
- 50% of solid foods and 25% of non-milk beverages are processed foods
- processed solid foods contain 25% starch or starch derived dry matter and processed non-milk beverages contain 12% starch or starch derived dry matter
- the maximum glucoamylase level in final solid foods was 990 mg TOS/kg (based on the level in flour) and for non-milk beverages was 929 mg TOS/kg starch (i.e. the highest use level from all uses within each group).

Based on these assumptions, the applicant calculated the TMDI of glucoamylase to be 5.88 mg TOS/kg body weight/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. This is the standard level used in a budget method calculation where there is potential for the enzyme to be in baby foods or general purpose foods that would be consumed by infants (Hansen, 1966), which for this enzyme would be from the bakery products, glucose syrups and other starch hydrolysates.
- FSANZ would generally 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 based on FSANZ's calculations for solid foods and non-milk beverages were 6.19 mg TOS/kg body weight/day and 2.79 mg TOS/kg body weight/day respectively, resulting in a total of 8.97 mg TOS/kg bw/day.

Both the FSANZ and applicant's estimates of the TMDI will be overestimates of the dietary exposure given the conservatism in the budget method. This includes that it was assumed that the enzyme remains in the final foods and beverages. 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.

4 Discussion and Conclusion

FSANZ concludes that the proposed use of this protein engineered variant of glucoamylase

as a processing aid in baking processes, brewing processes and starch processing for the production of 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 protein engineered glucoamylase from modified *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: *P. oxalicum*) was not genotoxic *in vitro*. The NOAEL in a 13-week oral gavage study in rats was 1360 mg TOS/kg bw/day, the highest dose tested. The TMDI was calculated as 8.97 mg TOS/kg bw/day. Comparison of the NOAEL and the calculated TMDIs gives a Margin of Exposure (MOE) of around 150.

No significant homology to known food allergens was identified. Some homology to a respiratory allergen from *S. commune* (splitgill mushroom) was identified, however respiratory allergens are not usually food allergens and *S. commune* is consumed as a food overseas without reports of food allergy. Based on the available evidence the enzyme is unlikely to pose a food allergenicity concern.

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.

5 References

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