FOOD STANDARDS Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

9 March 2023
234-23

Supporting document

Risk and Technical Assessment – Application A1245

Alpha-glucosidase from GM *Trichoderma reesei* as a processing aid in brewing

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Executive summary

Danisco New Zealand Limited (Danisco) has applied to Food Standards Australia New Zealand to amend the Australia New Zealand Food Standards Code (the Code) to permit an additional use of alpha-glucosidase, as a processing aid in the brewing of beer. The enzyme alpha-glucosidase is produced from a genetically modified (GM) strain of *Trichoderma reesei* containing the alpha-glucosidase gene from *Aspergillus niger*.

FSANZ previously assessed this enzyme under Application A1169 (Alpha-Glucosidase from *Trichoderma reesei* as a processing aid (Enzyme)) for use as a processing aid in the manufacture or processing of various foods but not including the brewing of beer. FSANZ has undertaken a further assessment to determine whether the enzyme achieves the proposed technological purposes in the brewing of beer and to evaluate public health and safety concerns that may arise from extending the use of the enzyme as proposed.

FSANZ concludes that the proposed use of this enzyme as a processing aid in brewing of low alcohol and lower carbohydrate beer is consistent with its typical functions of catalysing the transfer of glycosyl units and hydrolysis releasing glucose, respectively. The enzyme can be added during the mashing step to produce a higher proportion of non-fermentable sugars which reduces fermentability therefore producing low alcohol beer. It can also be added during the fermentation stage to reduce the non-fermentable carbohydrates thereby increasing the fermentable carbohydrates. This means more of the carbohydrates (as sugars) are fermented, leaving less in the final fermented beer.

Alpha-glucosidase performs its technological purpose during the production of food and is not performing a technological purpose in food for sale. It is therefore appropriately categorised as a processing aid for the purposes of the Code.

There are relevant identity and purity specifications for the enzyme in the Code.

No public health and safety concerns were identified in the assessment of this alpha-glucosidase produced from a GM strain of *T. reesei* under the additional proposed use. *T. reesei* has a long history of safe use as a production microorganism of enzyme processing aids, including several that are already permitted in the Code. The production organism is neither pathogenic nor toxigenic and the modification has been shown to be stably inherited.

An 18-week oral gavage study in rats has previously been reviewed by FSANZ establishing a no observed adverse effect level (NOAEL) of 77.2 mg total organic solids (TOS)/kg body weight (bw)/day, the highest dose tested. The theoretical maximum daily intake (TMDI) of this alpha-glucosidase for the refined scenario selected for the risk characterisation was 0.35 mg TOS/kg bw/day. A comparison of the NOAEL and the TMDI results in a Margin of Exposure (MOE) of over 200.

Updated bioinformatics searches found no significant homology with known toxins or allergens.

Nutrient raw materials used in the production of the applicant's alpha-glucosidase include glucose derived from wheat. Therefore the enzyme preparation may contain traces of wheat.

Based on the reviewed data it is concluded that, in the absence of any identifiable hazard, an Acceptable Daily Intake (ADI) of 'not specified' is still considered appropriate.

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1 Introduction

The applicant, Danisco New Zealand Limited (Danisco) has applied to Food Standards Australia New Zealand (FSANZ) to amend the Australia New Zealand Food Standards Code (the Code) to permit an additional use of alpha-glucosidase from *Trichoderma reesei* containing the alpha-glucosidase gene from *Aspergillus niger*. The additional use is as a processing aid in the brewing of brewed beverages, specifically low alcohol beer and lower carbohydrate beer.

FSANZ previously assessed this enzyme for use in various foods under Application A1169 – Alpha-Glucosidase from *Trichoderma reesei* as a processing aid (Enzyme). Following that assessment, FSANZ concluded that there were no health and safety concerns associated with the use of this enzyme. Alpha-glucosidase from *T. reesei* containing the alpha-glucosidase gene from *A. niger* was therefore approved for use as a processing aid for various purposes but not for brewing. FSANZ has referred to the assessment of A1169 where applicable in this report[1].

1.1 Objectives of the assessment

The objectives of this technical and risk 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 issues that may arise from the use of this enzyme, as a processing aid in the brewing of beer, specifically by considering the:
  - safety and history of use of the production organism
  - safety of the enzyme.

2 Food technology assessment

2.1 Identity of the enzyme

The applicant provided relevant information regarding the identity of the alpha-glucosidase enzyme. FSANZ verified this using the IUBMB[2] enzyme nomenclature database (McDonald et al 2009) and information in relation to a generally recognized as safe (GRAS) notice as detailed in Section 2.3 below. Details of the identity of the enzyme are provided in Table 1.

The alpha-glucosidase that is the subject of this application is produced by submerged fermentation of *T. reesei* containing the alpha-glucosidase gene from *A. niger.* Taking into account the production microorganism for this enzyme, FSANZ has confirmed that it is the same enzyme as that assessed under A1169 and for which permission for use for a range of purposes currently exists in the Code.

[1] Associated reports are available at https://www.foodstandards.gov.au/code/applications/Pages/A1169.aspx
[2] International Union of Biochemistry and Molecular Biology. EC 3.2.1.20 (qmul.ac.uk)

Table 1 *Identity*

Generic common name:	Alpha-glucosidase
Accepted IUBMB name:	α-glucosidase
Systematic name:	α-D-glucoside glucohydrolase
Other names:	maltase; glucoinvertase; glucosidosucrase; maltase-glucoamylase; α-glucopyranosidase; glucosidoinvertase; α-D-glucosidase; α-glucoside hydrolase; α-1,4-glucosidase
EC number[3]:	3.2.1.20
Reactions:	1. Hydrolysis of terminal, non-reducing (1→4)-linked α-D-glucose residues with release of α-D-glucose 2. Catalyses both the hydrolysis and transference of D-glucosyl units of oligosaccharides and the conversion of 1,4 glucosidic linkages to 1,6 glucosidic linkages

The hydrolysis reaction scheme for alpha-glucosidase is available under its record in the enzyme database BRENDA[4] (Chang et al 2021).

2.2 Manufacturing process

2.2.1 Production of the enzyme

The applicant stated that the manufacturing process for the enzyme as outlined in Appendix A of the application for A1169 remains the same. That manufacturing process follows standard industry practice for the manufacture of enzymes. It was summarised by FSANZ in the supporting document 1 for that A1169[5]. The process is therefore not detailed here.

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/WHO 2006) and in the Food Chemicals Codex (FCC 2020). These specifications are included in earlier publications of 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. In addition, under JECFA, enzyme preparations must meet the specifications criteria contained in the individual monographs. In the case of alpha-glucosidase, there is no individual monograph.[6]

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. Limits for lead and arsenic are already included in section S3—3, under Japan's Specifications and Standards for Food Additives (JSFA) (Japan Ministry of Health, Labour and Welfare)[7].

[3] Enzyme Commission, internationally recognised number that provides a unique identifier for enzymes
[4] Information on EC 3.2.1.20 - alpha-glucosidase - BRENDA Enzyme Database (brenda-enzymes.org)
[5] Available on the FSANZ website at Application A1169
[6] 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/
[7] Section D. monographs. Alpha-glucosidase. Accessed 21 December 2022

The applicant stated that appropriate good manufacturing practice (GMP) controls and processes are used in the manufacture of their alpha-glucosidase to ensure the finished preparation does not contain impurities of a hazardous or toxic nature. They provided the results of analysis of four different batches of their alpha-glucosidase enzyme. Table 2 provides a comparison of the analyses with international specifications established by JECFA, the FCC and the Japan Ministry of Health. The results also showed that the enzyme met the maximum levels for cadmium and mercury (1 mg/kg) in section S3—4. Based on these results, the enzyme met the relevant specifications in Schedule 3 of the Code.

Table 2 *Comparison of the manufacture's alpha-glucosidase enzyme preparation compared to JECFA, Food Chemicals Codex, and the Japan specification for enzymes*

Analysis	Analysis provided by applicant	Specifications		
		JECFA (2006)	Food Chemicals Codex (FCC 2020)	JSFA (Japan), 9th Edition
Lead (mg/kg)	<0.01	≤5.0	≤5.0	≤5.0
Arsenic (mg/kg)	<0.01	-	-	≤3.0
Total coliforms (cfu/g)	<1	≤30	≤30	-
Salmonella (in 25 g)	Negative	Absent	Negative	Negative
E. coli (in 25 g)	Negative	Absent	-	Negative
Antimicrobial activity	Negative	Absent	-	-

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. Refer to Section 3.5 below for the total organic solids (TOS) value. The recommended use levels in brewing provided by the applicant are 40-60 mg TOS/kg of raw material (cereals).

2.3 Technological purpose of the enzyme

The applicant has requested permission to use alpha-glucosidase from GM *T. reesei* as a processing aid in brewing to produce beer, as an extension to the current permissions provided following assessment of the earlier application, A1169.

The applicant has received a No Questions letter from the United States Food and Drug Administration (FDA) to a generally recognized as safe (GRAS) notification for the same enzyme as in this application. This was for the GRAS Notice (GRN) 703[8]. The technological purpose in GRN 703 was essentially identical to FSANZ's earlier assessment of the same enzyme under A1169. Within GRN 703 the applicant noted that the alpha-glucosidase enzyme from the same production organism contains both alpha-glucosidase activity (a hydrolysis reaction) and transglucosidase activity (a transfer reaction) and so two technological functions.

[8] Available at https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=703

Based on sequence identity, the gene encoding for that enzyme is identified in Genbank as *aglA* (Genbank accession: D45356.1) and the scientific name is alpha-glucosidase (GenBank: BAA23616.1), even though functionally this enzyme also displays transglucosidase activity (Duan et al, 1995; Nakamura et al, 1997, Saman et al 2008).

The applicant stated that it's alpha-glucosidase performs two specific technological functions which are quite different to each other depending on when it is added during brewing and how it is utilised. Specifically, it can be added either:

- during mashing to produce a higher proportion of non-fermentable sugars which reduces fermentability of the wort produced and so produces lower alcohol beer (utilising the transfer reaction)
- during fermentation to increase the proportion of fermentable sugars so more of the carbohydrates (sugars) are fermented, leaving less carbohydrates in the final fermented beer, producing a lower carbohydrate beer (utilising the hydrolysis reaction).

These functions are further discussed in Section 2.4 below.

The *Guidelines on Substances used as Processing Aids* (CAC/GL 75-2010) sets out general principles for the safe use of substances used as processing aids. The guideline states that substances used as processing aids shall be used under conditions of GMP. Therefore, use of commercial enzyme preparations should follow GMP, where use is at a level that is not higher than that necessary to achieve the desired enzymatic reaction.

The applicant provided information on the physical and chemical properties of the enzyme preparation. Table 3 summarises this information.

Table 3 *Physical and chemical properties of alpha-glucosidase enzyme preparation*

Physical and chemical properties of commercial enzyme preparation	
Appearance	Brown liquid
Temperature optimum for maximum activity	58-70°C, with activity observed from 30-90°C
Thermostability	Relatively stable for 30 minutes at 60°C, inactivated after 30 minutes of incubation at 71°C
pH optimum	Below approximately 5.6, exhibits activity at <8.3.

2.4 Technological justification

For the extension of use of the enzyme for use in the brewing, the two distinct reactions catalysed by alpha-glucosidase; transfer and hydrolysis, are used separately in the production of two distinct type of beers as follows.

2.4.1 Transfer reactions used in the mash to produce low alcohol beers

For this process, the enzyme transfers glycosyl units (essentially cleaved off glucose molecules) to existing sugars produced during the mashing process. The outcome of the transfer reaction is to increase the size of the released carbohydrates (sugars) therefore increasing the proportion of non-fermentable sugars that the yeast will not be able to utilise in the subsequent fermentation. This is further detailed below.

As explained by the applicant, adding the enzyme to the mash with maltose present will produce larger and more complex sugars such as isomalto-oligosaccharides (IMO) which are not fermentable by brewers yeast. The process usually cleaves a glucose molecule from a sugar or dextrin molecule where there is a 1,4- bond to the next glucose unit, typically maltose and maltotriose as well as panose, in brewing. The removed and transferred glucose molecule is connected to another sugar molecule in a 1,6 bond, rendering it non-fermentable and reducing the proportion of fermentable sugars in the wort. The outcome is to reduce the fermentability of the produced wort and so produce a lower alcohol beer.

2.4.2 Hydrolysis reactions used during fermentation to produce lower carbohydrate beers

When the enzyme is added during fermentation at the point where maltose has been fermented, alpha-glucosidase acts to produce glucose and reduce the amount of non-fermentable sugars in the product. This ensures that a large proportion of the carbohydrates in the fermentation broth are able to be fermented by yeast leaving a reduced proportion of carbohydrate in the resultant beer. During fermentation the yeast will consume all maltose, therefore, for optimal performance it is suggested by the applicant that the enzyme is added after the maltose has been consumed by the yeast.

2.5 Food technology conclusion

FSANZ concludes that the proposed use of this enzyme as a processing aid in brewing of low alcohol and lower carbohydrate beer is consistent with its typical functions of catalysing the transfer of glycosyl units and hydrolysis releasing glucose, respectively. The enzyme can be added during the mashing step to produce a higher proportion of non-fermentable sugars which reduces fermentability of the wort produced and so produces low alcohol beer. It can also be added during fermentation to reduce the non-fermentable carbohydrates and increase the fermentable carbohydrates (sugars). This means more of the carbohydrates (as sugars) are fermented, leaving less in the final fermented beer.

Alpha-glucosidase performs its technological purpose during the production of food and is not performing the technological purpose in the food for sale. It is therefore appropriately categorised 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 their enzyme meets these specifications.

3 Safety assessment

3.1 Objective for safety assessment

The objective of this safety assessment is to evaluate any potential public health and safety concerns that may arise from extending the use of *A. niger* alpha-glucosidase produced using GM *T. reesei* as a processing aid.

3.2 History of use

3.2.1 Host organism

T. reesei is a common soil fungus that was initially isolated from deteriorating canvas made from cellulosic material. The original isolate QM6a is the type strain for *T. reesei* (Olempska-Beer *et al.,* 2006). *T. reesei* QM6a strains are non-pathogenic, not known to possess any

virulence factors associated with colonisation or disease, and do not present any human toxicity concerns (US EPA 2012). Several review papers support the safety of *T. reesei* QM6a strains with no production of known mycotoxins or antibiotics under conditions used for enzyme production (Nevalainen et al., 1994; Blumenthal 2004; Kubicek et al., 2007; Frisvad et al., 2018). *T. reesei* QM6a strains are known to produce peptaibol antibiotic paracelsin, but industry-standard submerged fermentation conditions are not linked to the production of paracelsin (US EPA 2012).

FSANZ has previously approved alpha-glucosidase from *T. reesei* following assessment of A1169. FSANZ has also previously assessed the safety of *T. reesei* as the source organism for a number of other enzyme processing aids. Schedule 18 lists the following permitted enzymes derived from *T. reesei:* cellulase, endo-1,4-beta-xylanase, β-glucanase, hemicellulase multicomponent enzyme and polygalacturonase or pectinase multicomponent enzyme.

3.2.2 Gene donor organism(s)

The alpha-glucosidase gene was sourced from *A. niger*, a filamentous fungus ubiquitous in the environment. FSANZ has previously assessed the safety of *A. niger* as a gene donor for glucose oxidase and also as the source organism for at least 28 endogenous enzyme products, including alpha-glucosidase.

3.3 Characterisation of the genetic modification(s)

The full details of the genetic modification were provided and assessed for A1169, but cannot be disclosed as they are confidential commercial information. Briefly, an expression cassette containing the alpha-glucosidase gene from *A. niger* and a selectable marker gene was inserted into the *T. reesei* genome using standard methodologies. The presence and stability of the inserted DNA was demonstrated by Southern blot analyses.

3.4 Safety of alpha-glucosidase

3.4.1 History of safe use of the enzyme

This enzyme has been used in the USA for production of IMO since 2009, and for production of organic acids and potable alcohol since 2014. The enzyme was approved as a processing aid in Australia and New Zealand in 2019.

FSANZ could not locate any reports of adverse health effects since alpha-glucosidase was approved for use in Australia and New Zealand in the scientific literature.

3.4.2 Bioinformatics concerning potential for toxicity

Bioinformatic analyses for toxin homology were evaluated as part of A1169, where BLAST searches were conducted against the UniProt[9] animal toxin database and the Uniprot annotated Protein Knowledge database. No sequence similarity with known protein toxins were observed.

FSANZ conducted an updated search (Nov 2022) using the alpha-glucosidase amino acid sequence against the UniProt annotated protein knowledge database. No sequence similarity to known toxins was observed in the top 1000 hits supporting the finding of the original assessment.

[9] UniProt database: https://www.uniprot.org/

3.4.3 Toxicology studies

Two toxicity study reports were assessed by FSANZ as part of the A1169 risk and technical assessment. Both studies were conducted with the alpha-glucosidase enzyme that is the subject of this application.

An acute oral toxicity study in rats (Harlan Laboratories, 2009a), conducted in accordance with OECD test guideline (TG) 423, concluded that the acute oral median lethal dose for the enzyme in the rat was greater than 2000 mg/kg bw, based on the lack of mortality observed in the study.

An 18-week oral toxicity study in rats (Harlan Laboratories, 2010), meeting the study requirements of OECD TG 408, concluded that the no observed adverse effect level (NOAEL) was 77.2 mg TOS/kg bw/day, the highest dose tested.

3.4.4 Genotoxicity assays

Two genotoxicity study reports were assessed by FSANZ as part of the A1169 risk and technical assessment. Again, both studies were conducted with the alpha-glucosidase enzyme that is the subject of this application.

A bacterial reverse mutation assay (Harlan Laboratories, 2009b) conducted to OECD TG 471 concluded that the alpha-glucosidase test item was not mutagenic in the bacterial experimental strains, with or without metabolic activation.

An *in vitro* chromosomal aberration test conducted using human lymphocytes (Harlan Laboratories, 2009c) to OECD TG 471 concluded that the alpha-glucosidase test item did not induce chromosomal aberrations in human lymphocytes *in vitro.*

3.4.5 Potential for allergenicity

Bioinformatic analyses for allergenicity was evaluated as part of A1169, where the amino acid sequence of alpha-glucosidase was compared against the AllergenOnline[10]. No allergen sequences were identified using this analysis.

FSANZ undertook an updated allergen search (Nov 2022) performing a FASTA search comparing the mature amino acid sequence of alpha-glucosidase against the AllergenOnline database, using two sequence alignments: the full-length protein (E value[11] less than 0.1), and an 80 mer sliding window (more than 35% identity). No matches were identified using these search parameters.

The applicant notes that the manufacturing process of the alpha-glucosidase is the same as that considered under A1169, where the raw materials used for fermentation included glucose derived from wheat. The previous estimate provided by the applicant calculated that the highest amount of wheat protein in food processed with the enzyme would be 5 ppb.

3.4.6 Approvals by other regulatory agencies

The US FDA responded with a No Questions letter to a generally recognised as safe (GRAS) notification for this alpha-glucosidase, under the name transglucosidase in 2010, and under its current name in 2017. However, this is not an assessment by the FDA and not accepted

[10] AllergenOnline: http://www.allergenonline.org/

[11] The E value (or Expect value) indicates the significance of a match found when searching a sequence database. The closer an E value gets to zero, the less likely an alignment could have been produced by chance

by FSANZ as an assessment by an international agency.

3.5 Dietary exposure assessment

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 likely levels of dietary exposure, assuming that all of the TOS from the alpha-glucosidase 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). The method is used by international regulatory bodies and the JECFA (FAO/WHO 2021) for dietary exposure assessments for processing aids.

This alpha-glucosidase enzyme from the same source was approved by FSANZ for use as a processing aid in the manufacture or processing of potable alcohol, lysine, organic acids, monosodium glutamate and other biochemicals, isomalto-oligosaccharides and other sweeteners under the previous application, A1169. In this application, approval is sought for the use of this enzyme as a processing aid in brewing of beer. Hence, in assessing the dietary exposure to this enzyme, FSANZ has considered relevant data from both applications.

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

- the maximum physiological requirement for solid food (including milk) is 25 g/kg body weight/day
- 50% of solid food is processed
- 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)
- 25% of non-milk beverages are processed
- all non-milk beverages contain the highest use level of 60 mg TOS/kg in the raw material (cereals)
- a raw material to final food ratio of 0.17 kg cereals per litre of beer
- the densities of non-milk beverages are ~1
- all non-milk beverages contain the highest use level of 10.2 mg TOS/kg in the final food (this is a slightly higher concentration compared to that used in the DEA for A1169 as a result of the new proposed use for A1245)
- all of the TOS from the enzyme preparation remains in the final food.

Based on these assumptions, and summing the TMDI from both solid foods and non-milk beverages from A1169 and the TMDI for non-milk beverage portion of the calculation from A1245, the applicant calculated the TMDI of the TOS from the enzyme preparation to be 0.698 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 in two ways:

- Firstly, using the following assumptions that are highly 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 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.
  - The applicant assumed a raw material to final food ratio of 0.17 kg cereals per litre of beer, however, this ratio is variable. Hence FSANZ has used a higher value (0.28 kg cereals per litre of beer) as provided by the applicant to represent the worst-case scenario.
  - The concentration of alpha-glucosidase in solid final foods will not exceed the maximum level of 22.03 mg TOS/kg (the level used in the dietary exposure assessment for A1169) and for non-milk beverage final foods will not exceed the maximum level of 10.2 mg TOS/kg.

- Secondly, using the following assumptions that are also conservative, but more representative of actual food consumption patterns and are a second tier or refinement of the estimate of dietary exposure:
  - The maximum amount consumed of solid food (including milk) is 20 g/kg body weight/day and non-milk beverages is 30 g/kg body weight/day (based on the Australian 2011-12 National Nutrition and Physical Activity Survey (NNPAS) consumption data).
  - The applicant assumed a raw material to final food ratio of 0.17 kg cereals per litre of beer, however this ratio is variable. Hence FSANZ has used a higher ratio (0.28 kg cereals per litre of beer) as provided by the applicant to represent the worst-case scenario.
  - The concentration of alpha-glucosidase in solid final foods will not exceed the maximum level of 22.03 mg TOS/kg (the level used in the dietary exposure assessment for A1169) and for liquid final foods will not exceed the maximum level of 10.2 mg TOS/kg.

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 was 0.97 mg TOS/kg bw/day for the first tier calculation using consumption data based on physiological requirements. The TMDI for the second tier refined calculation based on actual consumption amounts was 0.35 mg TOS/kg bw/day. In the FSANZ calculations, the non-milk beverage portion of the calculation was from A1245 only as it was a worst case in terms of having the highest concentration for that part of the budget method calculation.

The second tier refined TDMI is closer to actual dietary exposure over a long period of time, or over a lifetime given it is based on actual total food and beverage consumption amounts from nutrition survey data.

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 of the TOS from the enzyme preparation remains in the final foods and beverages whereas the applicant has stated that the enzyme is likely to be removed or inactivated

during processing, and perform no function in the final food to which the ingredient is added.

4 Discussion

The alpha-glucosidase enzyme is already approved as a processing aid in the manufacture or processing of various foods. This application is to permit an additional use of alpha-glucosidase, as a processing aid in brewing of beer.

No public health and safety concerns were identified in the assessment of this alpha-glucosidase produced from a GM strain of *T. reesei* under the additional proposed use. *T. reesei* has a long history of safe use as a production microorganism of enzyme processing aids, including several that are already permitted in the Code. The production organism is neither pathogenic nor toxigenic and the modification has been shown to be stably inherited.

An 18-week oral gavage study in rats has previously been reviewed by FSANZ establishing a NOAEL of 77.2 mg TOS/kg bw/day, the highest dose tested. The TMDIs calculated by FSANZ under two scenarios were 0.97 and 0.35 mg TOS/kg bw/day. A comparison of the NOAEL and the TMDIs give MOEs of approximately 80 and over 200 respectively. It was determined that the most appropriate estimate to use for the risk characterisation for this application given it better reflects more realistic longer term dietary exposure is the second tier calculation which gives an MOE of over 200.

Updated bioinformatics searches found no significant homology with known toxins or allergens.

Nutrient raw materials used in the bacterial fermentation process to produce alpha-glucosidase include glucose derived from wheat. Therefore the enzyme preparation may contain traces of wheat.

5 Conclusion

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

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