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

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Supporting document

Risk and technical assessment – Application A1227

Alpha-arabinofuranosidase from GM *Trichoderma reesei* as a processing aid

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Executive summary

Novozymes Australia Pty Limited applied to Food Standards Australia New Zealand (FSANZ) to amend Schedule 18 of the Australia New Zealand Food Standards Code (the Code) to include alpha-arabinofuranosidase (EC 3.2.1.55) as a processing aid for use in grain processing and potable alcohol production. The enzyme is sourced from a genetically modified (GM) strain of *Trichoderma reesei (T. reesei)* containing the alpha-arabinofuranosidase gene from *Talaromyces pinophilus.*

FSANZ has undertaken an assessment to determine whether the enzyme achieves its technological purpose in the quantity and form proposed to be used and to evaluate any public health and safety concerns that may arise from the use of this enzyme.

FSANZ concludes that the proposed use of alpha-arabinofuranosidase in grain processing and potable alcohol production is technologically justified for use at levels consistent with GMP. Analysis of the evidence provides adequate assurance that the use of this enzyme, in the quantity and form proposed is justified.

No public health and safety concerns were identified in the assessment of alpha-arabinofuranosidase produced by this GM *T. reesei* under the proposed use conditions. *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. Analysis of the modified production strain confirmed the presence and stability of the inserted DNA. Bioinformatics analysis indicated that the produced alpha-arabinofuranosidase does not have substantial homology with known toxins or food allergens.

Toxicity testing of the alpha-arabinofuranosidase enzyme showed no evidence of genotoxicity *in vitro* and the no observed adverse effect level (NOAEL) in a 90-day oral toxicity study in rats was the highest dose tested, 1116 mg total organic solids (TOS)/kg body weight (bw)/day. The theoretical maximum daily intake (TMDI) was calculated to be 0.33 mg TOS/kg body weight/day. Comparison of the NOAEL and the TMDI results in a margin of exposure (MOE) of around 3400.

In the absence of any identifiable hazard an Acceptable Daily Intake (ADI) ‘not specified’ is

appropriate.

Table of contents

1. Introduction

Novozymes Australia Pty Limited (Novozymes) applied to Food Standards Australia New Zealand (FSANZ) to permit the use of the enzyme, alpha-L-arabinofuranosidase (EC 3.2.1.55) as a processing aid for use in grain processing and potable alcohol production.

This enzyme is sourced from a genetically modified (GM) strain of *Trichoderma reesei (T. reesei)* containing the alpha-arabinofuranosidase gene from *Talaromyces pinophilus (T. pinophilus).* The host organism was developed from *T. reesei* RUT-C30 and derived from the original isolate from QM6a (ATCC 13631, Seidl et al., 2008).

1.1 Objectives of the assessment

Schedule 18 of the Australia New Zealand Food Standards Code (the Code) contains permissions for enzymes of microbial origin including alpha-arabinofuranosidase from *Aspergillus niger*. There is currently no permission in section S18-9 for alpha-arabinofuranosidase produced by a GM strain of *T. reesei* containing the gene for alpha-arabinofuranosidase from *T.pinophilus.* An assessment is required before permission can be considered for its use as a processing aid.

The objectives of this risk and technical assessment were to:

- determine whether the enzyme provides the proposed 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 enzyme as a processing aid, specifically by considering the
  - history of use of the gene donor and production microorganisms
  - characterisation of the genetic modification(s)
  - safety of the enzyme.

2 Food technology assessment

2.1 Characterisation of the enzyme

The production microorganism of the alpha-arabinofuranosidase enzyme is a GM strain of *T. reesei.* The applicant provided information regarding the identity of the alpha-arabinofuranosidase enzyme. FSANZ verified this by using the IUBMB[1] enzyme nomenclature database (McDonald et al 2009). Details of the identity of the enzyme are provided in Table 1.

[1] International Union of Biochemistry and Molecular Biology

2.1.1 Identity of the enzyme

Table 1 Identity

Generic common name:	arabinofuranosidase
Accepted IUBMB name:	Non reducing end α-L-arabinofuranosidase
Systematic name:	α-L-arabinofuranoside non-reducing end α-L-arabinofuranosidase
Other names:	arabinosidase; α-arabinosidase; α-L-arabinosidase; α-arabinofuranosidase; polysaccharide α-L-arabinofuranosidase; α-L-arabinofuranoside hydrolase; L-arabinosidase (ambiguous); α-L-arabinanase
EC number:	3.2.1.55
Reaction:	Catalyses the hydrolysis of terminal non-reducing α-L-arabinofuranosidase residues in α-L-arabinosides.

The hydrolysis reaction scheme for alpha-arabinofuranosidase is available under its record in the enzyme database BRENDA[2] (Chang et al 2021).

2.2 Manufacturing process

2.2.1 Production of the enzyme

Novozymes' alpha-arabinofuranosidase is produced by submerged fermentation of a *T. reesei* microorganism expressing an alpha-arabinofuranosidase enzyme from *T. pinophilus.* The manufacturing process begins with fermentation followed by formulation and then quality control for the enzyme preparation. The fermentation steps include preparation of the inoculum, seed fermentation followed by the main fermentation. A recovery stage then includes multiple steps to separate, concentrate and stabilise the enzyme. Finally, the enzyme is blended into an enzyme preparation.[3] Novozymes' alpha-arabinofuranosidase enzyme preparation is sold as a liquid product consisting of water, sucrose, the enzyme solids, sodium benzoate and potassium sorbate.

The applicant states that the enzyme is manufactured in accordance with current Good Manufacturing Practice for Food (cGMP) and the principles of Hazard Analysis and Critical Control Point (HACCP). Novozymes follow relevant EC regulations for manufacture and packaging of food enzymes and for control and inspection. The quality management system complies with ISO9001:2015 for the development, production and sale of industrial enzymes.

The applicant states that all raw materials used in the production of the enzyme are of food grade quality that comply with relevant specifications.

Details on the manufacturing process, raw materials and ingredients used in the production of the alpha-arabinofuranosidase enzyme preparation were provided in the application or as Confidential Commercial Information (CCI).

[2] https://www.brenda-enzymes.org/enzyme.php?ecno=3.2.1.55

[3] Enzymes are generally sold as enzyme preparations, which consist of the enzyme(s) and other ingredients, to facilitate their storage, sale, standardisation, dilution or dissolution.

2.2.2 Allergen considerations

The applicant provided the Product Data Sheet for the enzyme preparation. This states that certain allergens are not present including cereals containing gluten, crustaceans, egg, fish, lupin, milk (including lactose), molluscs, nuts, peanuts, sesame, soy, sulphur dioxide/sulphites. The applicant also sent additional information to FSANZ as CCI, providing supporting information regarding the absence of the identified allergens in their enzyme preparation.

2.2.3 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) in its Compendium of Food Additive Specifications (FAO/WHO 2006) and in the Food chemicals codex (FCC 2008). 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. The applicant states that the final enzyme preparation is consistent with the requirements in both specifications. 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 representative batches of the alpha-arabinofuranosidase enzyme preparation. Table 2 provides a comparison of this analysis with international specifications established by JECFA and in the Food chemicals codex, as well as those in the Code (as applicable). Based on these results, the enzyme preparation met all relevant specifications for arsenic, metals and the microbiological criteria.

Table 2 Comparison of Novozymes alpha-arabinofuranosidase enzyme preparation compared to FAO/WHO JECFA, Food chemicals codex and Code specifications for enzymes

Analysis	*Analysis provided by manufacturer*	*FAO/WHO JECFA (2006)*	*Specifications Food Chemicals Codex (FCC, 2020)*	*Australia New Zealand Food Standards Code (section S3—4)*
Lead (mg/kg)	<0.5	≤5	≤5	≤2
Arsenic (mg/kg)	<0.3	-	-	≤1
Cadmium (mg/kg)	<0.05	-	-	≤1
Mercury (mg/kg)	<0.05	-	-	≤1
Total Coliforms (CFU/g)	<4	≤30	≤30	-
Enteropathogenic *E. coli* (in 25 g)	Not detected	Absent	-	-
***Salmonella* spp. (in 25 g)**	Not detected	Absent	Negative	-
Antimicrobial activity	Not detected	Absent	-	-

While the manufacturing process ensures the production microorganism, *T. reesei* is absent from the final enzyme preparation, the food enzyme 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 2.3 for the total organic solids (TOS) value.

2.3 Technological purpose of the enzyme

Alpha-arabinofuranosidase as requested by the applicant is intended for use in grain processing and potable alcohol production. Other functions related to food manufacturing include hydrolysis of arabinofuranosylglucosides to improve aroma in winemaking and clarification and thinning of juices.

The enzyme performs its technological function by hydrolysing arabinosidic linkages in arabinoxylan chains present in grains.

Hemicelluloses are found in plant cell walls including grains. L-Arabinosyl residues including arabinoxylan, arabinan, gum arabic and arabinogalactan are found in hemicelluloses. With endo-β-1,4-xylanase, β-xylosidase and other accessory enzymes, alpha-arabinofuranosidase works synergistically to enable the conversion of these residues to arabinose, a fermentable sugar (Saha B., 2000).

Information provided by Novozymes on the physical and chemical properties of their enzyme preparation is summarised in Table 3.

Table 3 Physical and chemical properties of the alpha-arabinofuranosidase enzyme preparation

Physical and chemical properties of commercial enzyme preparation	
Enzyme activity	100 ARXU(M)/g*
Appearance	Amber coloured liquid
Storage conditions	0–10°C
Density	1.20 g/mL
Viscosity	1-25 cps

**ARXU(M)/g is alpha-arabinofuranosidase exo monosubstituted units per gram*

The enzyme preparation is available as a liquid concentrate standardised in alpha-arabinofuranosidase exo monosubstituted units to an activity of 100 ARXU(M)/g. The application includes the method to determine enzyme activity. To summarise, alpha-arabinofuranosidase catalyses arabinose release when wheat arabinoxylan found in grains is catalysed by galactose mutarotase. The beta-L-arabinose is oxidised by NAD+ to L-arabonic acid when beta-galactose dehydrogenase is present at pH 8.6. The amount of NADH formed is proportional to the amount of L-arabinose and measured by the increase in absorbance at 340nm.

The highest dosage level used during grain processing and potable alcohol production for solid and liquid foods is 90 ARXU(M)/kg grains or 36mg TOS/kg dry starch matter.

The Codex guideline, *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

good manufacturing practice (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 has mentioned that use of the enzyme is at GMP levels.

Alpha-arabinofuranosidase from *Aspergillus niger* is already permitted to be used as a processing aid by the Code, but not from *T. reesei* containing the alpha-arabinofuranosidase gene from *T. pinophilus* as requested by the applicant

2.4 Technological justification

As outlined in section 2.3, the technological purpose for use of alpha-arabinofuranosidase is in grain processing and potable alcohol production where it hydrolyses arabinoxylans in the grain to arabinose. The enzyme is denatured by heat during processing and removed by washing grains or distillation for potable alcohol. It therefore functions as a processing aid for the purposes of the Code.

Although grain processing and potable alcohol production can occur without the use of enzymes there are advantages for production of these products. Benefits of alpha-arabinofuranosidase in grain processing include increased gluten and starch yield and more efficient removal of water, due to its efficiency in degradation of arabinoxylans in the grain fibre.

Benefits associated with the use of alpha-arabinofuranosidase in potable alcohol production include a high solids concentration during mashing. This increases fermentable sugars following hydrolysis of arabinoxylans. There are also production efficiencies, including improved heat exchange, centrifugal separation and mass transfer in fermentation.

2.5 Food technology conclusion

FSANZ concludes that the proposed use of alpha-arabinofuranosidase as an enzyme in grain processing and potable alcohol production is consistent with its technological purpose of catalysing the hydrolysis of arabinoxylans in grain to arabinose. FSANZ concludes that the information included in the application and assessment support the proposed use. The use of the enzyme, in the form and requested amount at a level consistent with GMP is technologically justified and has been demonstrated to be effective in achieving its stated purpose.

Alpha-arabinofuranosidase performs its technological purpose during the production and processing 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

The objective of this safety assessment was 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

3.1.1 Host organism

T. reesei is a common, hypercellulolytic, 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) and has been registered with the American Type Culture Collection under ATCC13631. Strain QM6a is the wild type of practically all *Trichoderma reesei* industrial production strains (Nevalainenet et al., 1994). Due to the secretion of a range of cellulolytic enzymes, this fungus has been used since the 1980s for the industrial production of enzymes for a range of industries including food (Nevalainen and Peterson, 2014; Paloheimo et al., 2016).

Several review papers support the safety of *T. reesei* with no production of known mycotoxins or antibiotics under conditions used for enzyme production (Nevalainen et al., 1994; Kubicek et al., 2007; Peterson and Nevalainen, 2012; Frisvad et al., 2018). It is listed as Risk Group 1 in the microorganism classification lists of the German Federal Institute for Occupational Safety and Health (BAuA, 2016) and meets the requirements of a Biosafety Level 1 organism based on the Biosafety in Microbiological and Biomedical Laboratories guidelines[4].

FSANZ has previously assessed the safety of *T. reesei* as the source organism for 15 processing aids in Schedule 18. The *T. reesei* production strain in this application was developed from the well-known type strain QM6a. The *T. reesei* strain RUT-C30 (ATCC 56765) was developed from QM6a. The RUT-C30 strain is well known and documented (Peterson and Nevalainen 2012). The recipient strain used in the construction of the *T. reesei* production strain was derived from the RUT-C30 parental strain by a series of classical mutagenesis steps and spontaneous mutations. Data confirming the identity of the recipient strain was provided in the application.

The production organism is absent from the final enzyme preparation, the manufacturing process involves appropriate controls to prevent microbial contamination, and the microbial quality of the final enzyme preparation meets the specifications required by JECFA[5]. The production strain stability during fermentation was analysed using phenotypic parameters with no instability observed.

3.1.2 Gene donor organisms

The donor for the arabinofuranosidase gene is *Talaromyces pinophilus*.

This organism meets the criteria for a Biosafety Level 1 agent and is not associated with disease in healthy human adults.

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 a gene encoding the alpha-arabinofuranosidase enzyme was introduced into the genome of the host strain, *T. reesei*. The wild type alpha-arabinofuranosidase gene is derived from *T. pinophilus* genomic DNA and placed under the

[4] https://www.cdc.gov/biosafety/publications/bmbl5/index.htm
[5] https://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/enzymes/en/

control of a *T. reesei* promoter and terminator. Data provided by Novozymes and analysed by FSANZ confirmed the identity of the alpha-arabinofuranosidase enzyme.

A hybrid *Saccharomyces cerevisiae*/*Escherichia coli* vector containing the alpha-arabinofuranosidase expression cassette was used to transform the host strain. The expression cassette was integrated at specific integration sites in the host's genome and the final production strain was selected based on rapid growth and high alpha-arabinofuranosidase activity.

3.2.2 Characterisation of inserted DNA

Southern blot data provided by Novozymes and analysed by FSANZ confirmed the presence of the inserted DNA in the production strain. The applicant also provided the results of genome sequencing 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 gene, the applicant provided phenotypic data from large-scale fermentation of the production strain. These data confirmed that the gene encoding the alpha-arabinofuranosidase enzyme is expressed over multiple generations and is stable.

3.3 Safety of alpha-arabinofuranosidase from *T. reesei*

3.3.1 History of safe use

Alpha-arabinofuranosidase from *A. niger* is currently permitted as processing aid in Schedule 18 of the Code. However, alpha-arabinofuranosidase from *T. reesei* containing the gene for alpha-arabinofuranosidase from *T. pinophilus* is not permitted and does not have a history of safe use in Australia or New Zealand. The applicant stated that the enzyme is approved for use in Brazil, Denmark and Mexico.

There are no known reports of adverse effects arising from the consumption of *A. niger* alpha-arabinofuranosidase, nor *T. pinophilus* alpha-arabinofuranosidase, when used as a processing aid.

3.3.2 Bioinformatic assessment of enzyme toxicity

A Clustal alignment (ClustalW 2.0.10[6]) was performed by the applicant comparing the alpha-arabinofuranosidase protein sequence against all sequences in the UniProt[7] database (release: 2021-02-1) that contained the search word 'toxin' and not 'fragment'.
There were no toxin sequences identified with a sequence similarity to the enzyme above 20% identity, indicating that the alpha-arabinofuranosidase enzyme has no substantial homology with known protein toxins.

[6] Clustal W2 available from: http://www.clustal.org/clustal2
[7] UniProt database: https://www.uniprot.org

3.3.3 Evaluation of toxicity studies

A Clustal alignment (ClustalW 2.0.10[8]) was performed by the applicant comparing the alpha-arabinofuranosidase protein sequence against all sequences in the UniProt[9] database (release: 2021-02-1) that contained the search word 'toxin' and not 'fragment'.
There were no toxin sequences identified with a sequence similarity to the enzyme above 20% identity, indicating that the alpha-arabinofuranosidase enzyme has no substantial homology with known protein toxins.

3.3.3 Evaluation of toxicity studies

The *T. pinophilus* alpha-arabinofuranosidase test item used in the supplied toxicity studies was produced using GM *T. reesei* and was equivalent to the enzyme concentrate for commercialisation.

Animal studies

90-day repeated dose oral toxicity study in rats (Envigo CRS Limited, 2016). Regulatory Status: GLP; conducted according to OECD Test Guideline (TG) 408

The alpha-arabinofuranosidase test item was administered to Wistar Han rats (10/sex/group) at doses of 0, 112, 368 and 1116 mg TOS/kg body weight (bw)/day by oral gavage for 13 weeks. The vehicle control was water.

Animals were observed twice daily, with detailed observation performed twice weekly. Body weight, food consumption and detailed physical examinations for signs of toxicity were recorded weekly. Ophthalmological examination was conducted on all test animals prior to treatment and at study termination. Functional performance and sensory reactivity tests were performed in week 12. Gross pathology, haematology, clinical chemistry and measurement of organ weights was conducted on all animals at termination. Histopathological examination was conducted on the control and high-dose test groups only.

There was no mortality observed throughout the study period. No treatment-related effects were observed on feed consumption, body weights, haematology, clinical chemistry, ophthalmology, or functional observations (functional performance or sensory reactivity). No treatment-related macroscopic abnormalities or histopathological findings were observed in any of the test animals at necropsy.

The no observed adverse effect level (NOAEL) was 1116 mg TOS/kg bw/day, the highest dose tested.

Genotoxicity

Bacterial reverse mutation test (Covance Laboratories Ltd, 2016). Regulatory Status: GLP; conducted according to OECD TG 471

The potential mutagenicity of alpha-arabinofuranosidase was evaluated in *Salmonella enterica* ser. Typhimurium strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* strain WP2 *uvrA* pKM101, with and without metabolic activation using rat liver homogenate (S9). Bacterial cultures were treated for 1 hour with the test item between 16 – 5000 µg TOS/mL, before treatment was removed and bacterial cultures plated using a modified treat-and-plate

[8] Clustal W2 available from: http://www.clustal.org/clustal2
[9] UniProt database: https://www.uniprot.org

method. The experiment was conducted twice independently.

Positive controls in the absence of metabolic activation were 2-nitrofluorene (TA98), 4-nitroquinoline 1-oxide (TA100), *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (TA1535, WP2 *uvrA* pKM101) and ICR-171 (TA1537). The positive control in the presence of metabolic activation was 2-aminoanthracene (all strains). Water was used as the vehicle control.
No concentration-related increases in revertant colonies were observed in cultures treated with the test item, relative to vehicle controls, with or without metabolic activation. All positive control treatments showed the anticipated increases in mutagenic activity demonstrating the validity of the assay.

It was concluded that alpha-arabinofuranosidase was not mutagenic under the conditions of this test.

In vitro mammalian micronucleus test in human lymphocytes (Covance Laboratories Ltd, 2016). Regulatory status: GLP; conducted according to OECD TG 487

The potential of alpha-arabinofuranosidase to induce micronuclei formation in mammalian cells was tested using human lymphocytes isolated from peripheral blood, collected and pooled from two healthy female volunteers. Treatment with the alpha-arabinofuranosidase test item was either a 3-hour pulse exposure with or without S9, followed by a 21-hour recovery; or 24 hours exposure without S9, followed by 24-hour recovery. Positive control assays were conducted in parallel using mitomycin C as the clastogen positive control in the pulse treatment without S9, cyclophosphamide as the clastogen positive control in the pulse treatment with S9, and vinblastine as the aneugen positive control in the 24-hour exposure without S9. Sterile water was used as the vehicle control.

As a result of dose-selection experiments, the dose range for all treatments was 500 – 5000 µg TOS/mL.

There were no treatment related increases in the incidence of micronucleated human lymphocytes following exposure to the test item, relative to the vehicle controls. The positive controls demonstrated a statistically significant increase in micronuclei formation, validating the sensitivity of the experimental methodology. It was concluded that alpha-arabinofuranosidase was not clastogenic or aneugenic in human lymphocytes under the conditions of this study.

3.3.4 Potential for allergenicity

A FASTA search was performed comparing the amino acid sequence of the alpha-arabinofuranosidase against the AllergenOnline[10] database (queried February 2021) using four sequence alignments: the full-length protein (more than 35% identity), an 80 mer sliding window (more than 35% identity), a scaled 80 mer sliding window (more than 35% identity) and an 8 mer sliding window (100% identity). No allergen sequences were identified using these search parameters.

3.3.5 Assessments by other regulatory agencies

The *T. pinophilus* alpha-arabinofuranosidase produced using GM *T. reesei* is approved for use in Denmark, Mexico and Brazil. Reports of the assessments undertaken by these countries were not provided to FSANZ.

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

The US FDA responded with a “No Questions” letter to a GRAS Notification (GRN) 680. However, this is not an assessment by the FDA and not accepted by FSANZ as an assessment by an international agency.

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 ‘worst-case scenario’ approach to estimating likely levels of dietary exposure, assuming that all of the TOS from the alpha-arabinofuranosidase 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 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 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
- 50% of solid food is processed
- all processed solid food contains 25% grains (or grain-derived) dry matter
- 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 processed non-milk beverages contain 12% grain hydrolysates
- the densities of non-milk beverages are ~1
- all solid foods and non-milk beverages contain the highest use level of 36 mg TOS/kg in the raw material (grains)
- all of 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 0.22 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 assumption that is 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.

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 is and non-milk beverages is 0.33 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 of the TOS from the enzyme preparation remains in the final foods and beverages whereas the applicant has stated that it is likely to be removed during processing or diluted. In addition, the enzyme would be inactivated and perform no function in the final food to which the ingredient is added.

4 Discussion

No public health and safety concerns were identified in the assessment of alpha-arabinofuranosidase produced by this GM *T. reesei* under the proposed use conditions. *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. Analysis of the modified production strain confirmed the presence and stability of the inserted DNA. Bioinformatics analysis indicated that the produced alpha-arabinofuranosidase does not have substantial homology with known toxins or food allergens.

Toxicity testing of the alpha-arabinofuranosidase enzyme showed no evidence of genotoxicity *in vitro* and the NOAEL in a 90-day oral toxicity study in rats was the highest dose tested, 1116 mg TOS/kg bw/day. The TMDI based on FSANZ's calculations is 0.33 mg TOS/kg body weight/day. Comparison of the NOAEL and the TMDI results in a MOE of around 3400.

5 Conclusion

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.

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