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Te Mana Kounga Kai – Ahitereiria me Aotearoa

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Supporting document 1

Risk and technical assessment report – Application A1212

Beta-fructofuranosidase enzyme from *Aspergillus fijiensis*

Executive summary

Meiji Food Materia Co., Ltd applied to Food Standards Australia New Zealand (FSANZ) to amend Schedule 18 – Processing Aids of the Australia New Zealand Food Standards Code (the Code) to include *Aspergillus fijiensis* as a microbial source for the production of the approved processing aid beta-fructofuranosidase.

Methods for identifying microorganisms are constantly evolving and in some cases microorganisms will be re-identified as different species. In this application, a microbial source of beta-fructofuranosidase (EC number 3.2.1.26) currently approved in the Code was originally identified as the species *A. niger,* but advanced methods have now identified it as the species *A. fijiensis.*

The enzyme meets international purity specifications and has a long safe history of use in Australia and New Zealand.

No public health and safety concerns were identified in the assessment of beta-fructofuranosidase from *A. fijiensis* ATCC 20611 under the proposed use conditions. Based on the limited data available in the literature for the species *A. fijiensis* and as it has not previously been approved as an enzyme source in the Code, it is recommended that the source organism be designated at the strain level, rather than the species level. The identified strain, *A. fijiensis* ATCC 20611, is neither toxigenic nor pathogenic.

Beta-fructofuranosidase from *A. fijiensis* was not genotoxic *in vitro*. The no observed adverse effect level (NOAEL) in a 13-week repeated dose oral toxicity study in rats was the highest dose tested and corresponds to 920 mg/kg bw/day total organic solids (TOS). The theoretical maximum daily intake (TMDI) was calculated to be 0.52 mg/kg bw/day TOS for adults and 0.19 mg/kg bw/day for children. Comparison of the NOAEL and the calculated TMDIs gives a Margin of Exposure (MOE) of more than 1,700 for adults and 4,900 for children.

A low degree of homology was found between the beta-fructofuranosidase from *A. fijiensis* and peanut agglutinin precursor from *Arachis hypogaea*. Given the low degree of homology, the low levels likely to be found in foods, and the absence of case reports of food allergy to beta-fructofuranosidase, the risk of food allergy from the proposed uses of the enzyme is likely to be low.

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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1. Introduction

The application seeks permission to update the microbial source name for the currently permitted enzyme, beta-fructofuranosidase (Enzyme Commission (EC) number 3.2.1.26), as a processing aid in the Australia New Zealand Food Standards Code (the Code). That is, adding the updated name of the currently permitted source microorganism *Aspergillus niger*, being *Aspergillus fijiensis,* as a new permitted source microorganism for the enzyme.

The enzyme was permitted in the Code as a permitted processing aid as an outcome from application A1055[1] for the use of fructo-oligosaccharides (FOS) as a nutritive substance. It is noted that the permission for the enzyme is for all foods, for all technological purposes, not just for the production of FOS. The gazettal occurred in 2013. No other amendments to the permissions are sought. This assessment therefore needs to confirm if there has been any new information in the literature since gazettal to question the permission.

The purpose of the risk assessment and technical assessment of the application is also to:

- re-assess the safety of the enzyme and the source microorganism since the original permission
- check the updated name of the microorganism as requested is scientifically justified
- confirm the dietary exposure from the use of the enzyme does not indicate any safety concerns.

2 Food technology assessment

2.1 Characterisation of the enzyme

2.1.1 Identity and properties of the enzyme

The current production microorganisms of the permitted enzyme beta-fructofuranosidase are non-genetically modified strains of *Aspergillus niger* and *Saccharomyces cerevisiae*. A food technology section was written in the risk and technical assessment report for the original application which permitted the enzyme sourced from *A. niger* in 2013, being application A1055. That application was specifically related to short chain fructo-oligosaccharides, for which the enzyme is used in the production of. The current applicant was also the provider of the enzyme and technical details related to application A1055. This report summarises the information provided in the earlier document, which is still relevant.

Details of the identity of the enzyme are provided in Table 1.

Table 1: Identity and relevant details of the enzyme beta-fructofuranosidase

Generic common name:	beta-fructofuranosidase
Accepted IUBMB[2] name:	beta-fructofuranosidase β-fructofuranosidase
Systematic name:	β-D-fructofuranoside fructohydrolase

[1] Application A1055 - Short-chain Fructo-oligosaccharides
[2] International Union of Biochemistry and Molecular Biology

Other names:	invertase; saccharase; glucosucrase; β-h-fructosidase; β-fructosidase; invertin; sucrase; fructosylinvertase; alkaline invertase; acid invertase
EC number:	3.2.1.26
CAS[3] registry number:	9001-57-4
Reaction:	Hydrolysis of terminal non-reducing β-D-fructofuranoside residues in β-D-fructofuranosides
Optimal temperature (°C), (range)	50 – 60
Optimal pH, (range)	5.0 – 6.0 Negligible activity below 3.0 and above 10.0

2.2 Manufacturing process

2.2.1 Production of the enzyme

The enzyme is produced by a submerged fermentation process, which is the common production method of producing food enzymes. The specific processes are provided in the application which is summarised briefly here as these are very well known processes. Once fermentation is complete, the preparation is concentrated by filtration and freeze drying of the liquid enzyme preparation. Residual amounts of soybean material, which is used as a fermentation nutrient, may remain in the final enzyme preparation.

The manufacturing processes ensure the production microorganism is removed from the final enzyme preparation. The final enzyme preparation is produced to ensure it complies with international purity specifications of enzymes, being the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2006) and the Food Chemicals Codex (FCC) (USP, 2020) as discussed in the next section.

2.2.2 Specifications

There are international specifications for enzyme preparations used in food production (JECFA 2006; USP 2020). Both of these specification sources are primary sources listed in section S3—2 of the Code. Enzyme preparations must meet these specifications.

Table 2 provides a comparison of representative batch analysis of the enzyme preparation of this application with the international specifications established by JECFA and FCC, as well as those detailed in the Code (being section S3—4, as applicable).

[3] Chemical Abstracts Service

Table 2: Product specifications for commercial enzyme preparation

Analysis	Enzyme batch analysis	Specifications		
		JECFA	Food Chemicals Codex	Code
Lead (mg/kg)	≤ 5, ≤ 5, ≤ 5, ≤ 5	≤ 5	≤ 5	≤2[1]
Arsenic (mg/kg)	≤1, ≤1, ≤1, ≤1	-	-	≤1
Cadmium (mg/kg)	0.03, 0.03, 0.02, 0.03	-	-	≤1
Mercury (mg/kg)	≤0.01, ≤0.01, ≤0.01, ≤0.01,	-	-	≤1
Total coliforms (cfu/g)	<30,<30,<30,<30,	≤30	≤30	-
Salmonella (in 25 g)	Negative x4	Absent	Negative	-
Enteropathic *E. coli* (in 25 g)	Negative x4	Absent	-	-
Antimicrobial activity	Negative x4	Absent	-	-

1. Only applies if there are not relevant specifications in S3—2, i.e. JECFA or FCC specifications

Based on the above results, the enzyme preparation meets international and Code specifications for enzymes used in food production.

2.3 Food technology conclusion

The enzyme preparation meets international purity specifications. There are no food technological reasons to indicate the enzyme of this application differs to that currently approved.

3 Safety assessment

3.1 Objectives for safety assessment

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

- whether the updated name of the microorganism as requested is scientifically justified; and
- the history of use of the production microorganism; and
- the safety of the enzyme preparation.

3.2 History of use and identification of the microorganism

Aspergillus belonging to the *Nigri* section have a wide range of industrial applications in biomedicine, bioenergy, health and biotechnology (Vesth et al., 2018). A prominent example of the use of members of *Aspergillus* section *Nigri* is in the production of enzymes and organic acids through fermentation processes. As with many fungal species, the taxonomy of the *Aspergillus* genus has undergone a number of changes in recent years based on the emergence of new sequence data (D'hooge et al. 2018).

As indicated by the applicant, the initial deposit of this strain was conducted in association with the filing of a patent and was classified by the American Type Culture Collection (ATCC)

as *Aspergillus niger* ATCC® 20611™. The beta-fructofuranosidase enzyme produced by this organism has previously been approved for use in the Code through A1055 (FSANZ 2013), where the production strain is referred to by its previous name, *A. niger*. In November 1997, ATCC reclassified it as *A. japonicus* based on its morphology. This strain has now since been re-identified as *Aspergillus fijiensis* based on calmodulin gene sequencing conducted by the ATCC in 2015 with reference to Varga et al. (2011) who identified *A. fijiensis* as a new species.

However, since then, a number of references identified that *A. fijiensis* should be considered a synonym of the species *Aspergillus brunneoviolaceus* that was first described in 1955 (Batista & Maia 1955). For example, Hubka and Kolarik (2012) identified that β-tubulin primers are not specific for the *benA* gene for some taxa and preferentially amplify the *tubC* paralogue resulting in inaccurate identification. Hubka and Kolarik (2012) examined sequences that indicated that *A. brunneoviolaceus* should be considered a valid species. This species was not included in the study by Varga et al. (2011), and Hubka and Kolarik (2012) demonstrated low phylogenetic support for *A. fijiensis* separation from *A. brunneoviolaceus* as a separate species upon reanalysis. Hubka and Kolarik (2012) also stated the extrolite and secondary metabolite data do not clearly support *A. fijiensis* as a separated species and, as such, *A. fijiensis* should be treated as synonymous to *A. brunneoviolaceus*.

Subsequently, these findings were confirmed by Jurjević et al. (2012) providing further evidence supporting *A. fijiensis* as a synonym of *A. brunneoviolaceus* as they were indistinguishable by multilocus sequence analysis and belong in the same highly supported clade. Furthermore, *A. brunneoviolaceus* (syn. *A. fijiensis*) was referenced as the accepted species name by Samson et al (2014) who presented an updated accepted species list for the *Aspergillus* genus[4].

Calmodulin sequence data provided by the applicant from the ATCC was assessed by FSANZ and confirmed the species identity of the production strain as *A. brunneoviolaceus* (syn. *A. fijiensis*) according to accepted methodology presented by Samson et al (2014) involving comparison against verified calmodulin sequences of ex-type species. Due to the identification of an alternative species name to that presented by the applicant, an additional literature search was conducted for *A. brunneoviolaceus* in addition to *A. fijiensis.*

A. brunneoviolaceus (syn. *A. fijiensis*) are saprophytic airborne fungi belonging to the *Nigri* section, but are occasionally implicated in human pulmonary infection (Ezekiel et al 2020). However, no evidence of associated foodborne pathogenicity or toxicity were identified in the literature. Some strains of *Aspergillus* are capable of producing mycotoxins like ochratoxins, which are harmful to human health (Frisvad et al. 2011). *A. brunneoviolaceus* was not observed to produce ochratoxins or aflatoxins (Somma et al. (2012); Huang et al. (2020); Jurjević et al (2012)). As noted by the applicant, there are limited published studies that address the pathogenicity or toxigenic potential of organisms only identified as *A. fijiensis.*

The species *A. brunneoviolaceus* and *A. fijiensis* have not previously been approved for enzyme production and inclusion in the Code. They have a limited history of use in commercial enzyme production although the available data suggests these species present a low risk. It is recommended that the source organism be designated at the strain level rather than the species level.

The applicant has provided data demonstrating the absence of pathogenicity and toxicity of

[4] See also: current accepted species list https://www.aspergilluspenicillium.org

the strain *A. fijiensis* (ATCC 20611) through orally-inoculating laboratory mice with enzyme producing culture, culture supernatant, and culture precipitate. It is also noted that *A. fijiensis* (ATCC 20611) has been rated a Biosafety Level 1 organism based on the United States Public Health Service Guidelines[5]. Analysis results from four production batches provided by the applicant demonstrated mycotoxins, including ochratoxin A and aflatoxins, were not detected in the end product. The final product is tested in line with the Joint FAO/WHO Expert Committee on Food Additives (JECFA) requirements for microbially-derived enzyme preparations (JECFA 2006). The manufacturing process used by the applicant also includes appropriate controls to prevent unacceptable microbial contamination, and this was reflected in the batch analyses.

3.3 Safety of beta-fructofuranosidase

3.3.1 History of safe use of the enzyme

Beta-fructofuranosidase (EC 3.2.1.26) from *Aspergillus niger* (ATCC 20611) has been approved for use in Australia and New Zealand as a food processing aid for the production of FOS since 2013. Additionally, beta-fructofuranosidase from *Saccharomyces cerevisiae* is permitted as a food processing aid in Australia and New Zealand. Beta-fructofuranosidase from *A. fijiensis* (ATCC 20611) has been approved for use in Canada, France and Japan.

FSANZ's assessment of A1055 concluded that there were no safety concerns with the proposed use of beta-fructofuranosidase from *A. niger* as a processing aid in the production of FOS. FSANZ noted that the source organism has a long history of safe use in the production of food-grade enzymes, and neither the source organism or beta-fructofuranosidase are detectable in the final FOS preparation. An acceptable daily intake (ADI) of 'not specified' was considered appropriate.

Since FSANZ's previous assessment, new toxicity and genotoxicity studies with beta-fructofuranosidase from *A. fijiensis* (ATCC 20611) have become available, and are reviewed below.

3.3.2 Toxicology studies in animals

90-day repeated dose oral toxicity study in rats (BoZo Research Centre Inc., 2014). Regulatory Status: GLP; conducted according to OECD Test Guideline (TG) 408.

The test item was beta-fructofuranosidase, provided as a spray-dried powder that was 92% (w/w) total organic solids (TOS) and 10.8% (w/w) protein. The applicant confirmed that the enzyme tested in this study was representative of the commercial enzyme product.

Beta-fructofuranosidase was administered to Sprague-Dawley SPF Crl:CD(SD) rats (10 rats / sex / test group) at doses of 0, 100, 300 and 1000 mg/kg bw/day by oral gavage for 13 weeks. These doses were equivalent to 0, 92, 276 and 920 mg/kg bw/day TOS. The vehicle control was distilled water. Animals were housed individually with *ad libitum* access to food and water. Qualitative clinical observations were performed daily. Body weight recorded twice weekly and food consumption was recorded weekly. Detailed clinical observations were recorded weekly using a blinded observer. Ophthalmological examination was conducted on all test animals prior to treatment and to high-dose and control animals at study termination in week 13. Manipulative tests, grip strength and motor activity were undertaken in week 12. Urinalysis was performed in week 13 and haematological examination was conducted at necropsy. Gross pathology, measurement of organ weights and a histopathological examination was conducted on all animals at study termination.

[5] https://www.cdc.gov/biosafety/publications/bmbl5/index.htm

No mortality occurred during the study. No treatment related effects were observed in body weight, food consumption, manipulative testing, grip strength, motor activity, urinalysis, haematology, blood chemistry or ophthalmology examinations in any of the test animals. No macroscopic or histopathological changes related to treatment were observed at necropsy.

The no observed adverse effect level (NOAEL) was 1000 mg/kg bw/day, equivalent of 108 mg/kg bw/day of protein or 920 mg/kg bw/day TOS, which was the highest dose tested.

3.3.3 Genotoxicity assays

Two genotoxicity studies were submitted, a bacterial reverse mutation assay (Ames test) and an *in vitro* mammalian cell micronucleus test conducted on mouse lymphoma cells. Both experiments were conducted using beta-fructofuranosidase from *A. fijiensis* representative of the commercial product under review by FSANZ.

Bacterial reverse mutation test (BoZo Research Centre inc., 2014). Regulatory Status: GLP; conducted according to OECD TG 471.

The potential mutagenicity of beta-fructofuranosidase was evaluated in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* strain WP2 *uvrA*, with and without metabolic activation (S9 mix). Mutation tests were conducted twice independently, with three experimental replicates per treatment. The maximal test concentration of the beta-fructofuranosidase was 5000 µg/plate based on the findings of a preliminary dose range finding study.

Positive controls in the absence of metabolic activation were (*E*)-2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (WP2*uvrA*, TA98 and TA100), sodium azide (TA1535) and 2-methoxy-6-chloro-9-[3-(2-chloroethyl)aminopropylamino]acridine (TA1537). Positive controls in the presence of metabolic activation were 2-aminoanthracene (WP2*uvrA* and TA1535) and benzo[a]pyrene (TA98, TA100 and TA1537). Sterile deionized water was used as the vehicle control.

No concentration-related increases in revertant colonies were observed in cultures treated with beta-fructofuranosidase with or without metabolic activation. All positive control treatments showed significant increases in mutagenic activity, validating the integrity of the assay.

It was concluded that beta-fructofuranosidase was not mutagenic in any bacterial test strains under the conditions of this study.

In vitro mammalian cell micronucleus test BoZo Research Centre inc., 2014). Regulatory status: GLP; conducted according to OECD TG 487

The test was conducted on mouse lymphoma cells (L5178Y $tk^{+/-}$ -clone 3.7.2c). Metabolic activation was achieved through the addition of rat-liver hamogenate (S9). The experiment was conducted twice independently, where cells were either exposed to the test substance in the presence or absence S9 for 3 hours (short term), or exposed to the test substance for 24 hours (continuous) without S9. Positive control assays were conducted in parallel using mitomycin C as the clastogen positive control for the continuous treatment, cyclophosphamide as the clastogen positive control in the short time treatment with S9, and colchicine as the aneugen control in the short-term treatment without S9. 1000 cells per treatment were scored for each independent experiment.

Based on cytostatic activity observed in the dose selection experiments, the final test item concentrations were adjusted to 2500-4500 µg/mL for the 3 hour exposure without S9; 2000-5000 µg/mL for the 3 hour exposure with S9; and 500-2500 µg/mL for the 24 hour continuous exposure.

There were no increases in the incidence of micronucleated mouse lymphoma cells following exposure to the test item, relative to the vehicle controls, under the conditions tested. The positive controls demonstrated a statistically significant increase in micronuclei formation, validating the sensitivity of the experimental methodology.

It was concluded that the test beta-fructofuranosidase was not clastogenic or aneugenic in mouse lymphoma cells, under the conditions of this study.

3.3.4 Potential for allergenicity

The applicant provided details of an alignment search of the full-length beta-fructofuranosidase amino acid sequence against the AllergenOnline[6] database with an E-value[7] cut-off of 1.0 (October, 2014). A positive match was identified with peanut agglutinin precursor from *Arachis hypogaea*, demonstrating an E-value of 0.84 and 26.4% sequence identity. Given the low sequence similarity, this match was not deemed significant. A second search using an 80mer sliding window with a cut-off of > 35% homology did not identify any similarities with known allergens (January, 2015).

Given that both database searches were performed more than 24 months ago, FSANZ re-submitted both sequence searches (October, 2020), which confirmed that no recently added allergen sequences show similarity to the beta-fructofuranosidase sequence.

As noted in the food technology assessment, residual amounts of soybean material may remain in the final enzyme preparation. As part of application A1055, the applicant previously demonstrated that no beta-fructofuranosidase enzyme is carried into the final FOS product when used for the manufacture of FOS as permitted in the Code (FSANZ 2013). No other amendments to these permissions was sought.

3.3.5 Immobilisation agent

In some manufacturing practices, the beta-fructofuranosidase enzyme is immobilised before use in the FOS reaction. The applicant has provided documentation confirming that this immobilisation agent is compliant with European requirements for materials and articles intended to come into contact with food. The enzyme and immobilisation agent is removed completely from the final FOS product.

3.3.6 Assessments by other regulatory agencies

The applicant is currently applying for amended authorisations in multiple jurisdictions to reflect the updated taxonomic classification of the production strain.

The application includes a letter from Agence Nationale de Sécurité Sanitaire de l'Alimentation (ANSES) informing the applicant that the production strain of this enzyme will be changed in the annex of the order of October 19, 2006. The letter states that the subject beta-fructofuranosidase will now be referred to as "beta-fructofuranosidase from *Aspergillus niger* (synonyms *Aspergillus fijiensis*, *Aspergillus japonicus*) (ATCC 20611)".

[6] AllergenOnline: http://www.allergenonline.org/
[7] 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.

Health Canada have released a notice of modification (NOM/ADM-0144) to enable to use of *A fijiensis* as a source organism for invertase (beta-fructofuranosidase), reflecting updated scientific and taxonomic data. Health Canada's Food Directorate determined that an update to the source organism name did not require a pre-market safety assessment.

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 beta-fructofuranosidase food enzyme 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 processing aid concentration in foods and beverages, and the proportion of foods and beverages that may contain the processing aid. The TMDI can then be compared to an ADI or a NOAEL to estimate a margin of exposure for risk characterisation purposes.

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

- the beta-fructofuranosidase food enzyme is used as a processing aid in the production of the FOS ingredient only and is not added directly to food
- the maximum physiological requirement for solid food (including milk) is 50 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)
- 25% of solid food and 25% of non- milk beverages contain the enzyme for wide range of food categories for adults
- for young children, 100% of non- milk beverages contain the enzyme
- the maximum level of FOS in solid foods and non-milk beverages is 50%. For foods intended for young children the maximum use level of FOS is 6%. The maximum levels of FOS in solid foods and non-milk beverages used in the calculation of the TMDI were 500 g/kg for adults and 6 g/kg for young children
- the maximum beta-fructofuranosidase food enzyme level in FOS ingredient is 27.73 mg TOS/kg for solid foods and non-milk beverages
- all of the enzyme will remain in the final food, except for distilled beverages due to processing, however other uses in non-milk beverages were still assumed to contain the enzyme.

Based on these assumptions, the applicant calculated the TMDI of beta-fructofuranosidase food enzyme to be 0.52 mg TOS/kg body weight/day for adults and 0.02 mg TOS/kg body weight/day for young children.

As assumptions made by the applicant differ to those that FSANZ would have made in applying the budget method, FSANZ independently calculated the TDMI using the following

different assumptions that are conservative and reflective of a first tier in estimating dietary exposure:

- FSANZ used a maximum use level of FOS in solid foods and non-milk beverages of 500 g/kg for adults and 60 g/kg for children. These are equivalent to the assumption provided by the applicant that any food or beverage may contain a maximum use level of 50% FOS, and foods intended for young children may contain a maximum use level of 6% FOS.

- 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 25% 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 TDMI based on FSANZ's calculations for solid food and non-milk beverages were 0.17 mg TOS/kg body weight/day and 0.35 mg TOS/kg body weight/day respectively, a total of 0.52 mg TOS/kg bw/day for adults. While 0.02 mg TOS/kg body weight/day of solid foods and 0.17 mg TOS/kg body weight/day of non-milk beverages were estimated for young children with a total of 0.19 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 the enzyme remains in the final foods and beverages whereas the applicant has stated that it is likely to either be removed during processing or would be present in insignificant quantities, and 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 beta-fructofuranosidase from *A. fijiensis* (ATCC 20611) under the proposed use conditions, for the manufacture of all foods using any technological purpose.

The beta-fructofuranosidase that is the subject of this application has a history of safe use in Australia and other countries. The source species *A. brunneoviolaceus* and *A. fijiensis* have not previously been approved for enzyme production and inclusion in the Code. They have a limited history of use in commercial enzyme production although the available data suggests these species present a low risk. It is recommended that the source organism be designated at the strain level rather than the species level. The strain *A. fijiensis* (ATCC 20611) is neither toxigenic nor pathogenic.

Beta-fructofuranosidase from *A. fijiensis* was not genotoxic *in vitro*. The NOAEL in a 90-day oral gavage study in rats was the highest dose tested, 1000 mg/kg bw/day, equivalent of 108 mg/kg bw/day of protein or 920 mg/kg bw/day TOS. The TMDI was calculated as 0.52 mg/kg bw/day TOS for adults and 0.19 mg/kg bw/day TOS for children. Comparison of the NOAEL and the calculated TMDIs gives a Margin of Exposure (MOE) of more than 1,700 for adults and 4,900 for children.

A low degree of homology was found between the beta-fructofuranosidase sequence and peanut agglutinin precursor from *Arachis hypogaea*. Given the low degree of homology, the

low levels likely to be found in foods, and the absence of case reports of food allergy to beta-fructofuranosidase, the risk of food allergy from the proposed uses of the enzyme is likely to be low.

5 Conclusions

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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