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Te Mana Kounga Kai – Ahitereiria me Aotearoa

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Supporting Document 1

Risk and technical assessment – Application A1284 Triacylglycerol lipase from GM *Trichoderma reese*i as a processing aid

Executive summary

Food Standards Australia New Zealand (FSANZ) received an application from AB Enzymes GmbH to vary the Australia New Zealand Food Standards Code (the Code) to permit the use of a protein engineered triacylglycerol lipase from a genetically modified strain of *Trichoderma reesei* containing a gene from *Thermomyces lanuginosus* as a processing aid in the manufacture of bakery products and other cereal based products.

FSANZ concludes that the proposed use of this triacylglycerol lipase as an enzyme processing aid in the quantity and form proposed to be used, is consistent with its typical function of hydrolysing lipids. Triacylglycerol lipase performs its technological purpose during processing of food and does not perform its technological purpose in the food for sale, therefore functioning as a processing aid for the purposes of the Code. The enzyme meets relevant identity and purity specifications.

There are no safety concerns from the use of triacylglycerol lipase from GM *T. reesei*. Triacylglycerol lipases from other sources have a long history of safe use in food. The production organism is neither pathogenic nor toxigenic. Analysis of the GM production strain confirmed the presence and stability of the inserted DNA.

The Theoretical Maximum Daily Intake of the total organic solids from the triacylglycerol lipase preparation is 0.38 mg total organic solids/kg body weight. A comparison of the no-observed-adverse-effect-level and the theoretical maximum daily intake results in a Margin of Exposure of approximately 2,600.

Based on the reviewed data, in the absence of any identifiable hazard, an acceptable daily intake (ADI) 'not specified' is appropriate

Overall, FSANZ concludes that there are no safety concerns from the use of protein engineered triacylglycerol lipase from a genetically modified strain of *T. reesei* containing a gene from *T. lanuginosus*.

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1 Introduction

AB Enzymes GmbH has made an application to FSANZ seeking permission for the protein engineered enzyme triacylglycerol lipase (EC 3.1.1.3) from a genetically modified *Trichoderma reesei* (AR-822) containing a triacylglycerol lipase gene from *Thermomyces lanuginosusas* as a processing aid.

The enzyme would be used in the manufacture of:

- bakery products such as, but not limited to, bread, steamed bread, bread buns, tortillas, cakes, pancakes and waffles.
- other cereal-based products

There are several permissions for triacylglycerol lipase in the Australia New Zealand Food Standards Code (the Code). However, there is no permission for its use sourced from a genetically modified strain of *T. reesei,* containing the gene for triacylglycerol lipase isolated from *T. lanuginosusas*. Therefore, the use of this enzyme as a food processing aid requires pre-market assessment.

1.1 Objectives of the Assessment

The objectives of this safety assessment are to evaluate any potential public health and safety concerns that may arise from the food use of triacylglycerol lipase from GM *T. reesei* containing a triacylglycerol lipase encoding gene from *T. lanuginosusas.*

Some information relevant to this assessment is commercially confidential information (CCI), therefore some details cannot be provided in a public report.

2 Food Technology Assessment

2.1 Specifications for identity and purity

2.1.1 Identity

FSANZ verified the information about the identity of the enzyme from the application with the appropriate enzyme nomenclature reference (IUBMB (International Union of Biochemistry and Molecular Biology) 2017). Table 1 has a summary of the identity of triacylglycerol lipase.

Table 1 Identity

Systematic name:	Triacylglycerol acylhydrolase
Accepted IUBMB[1] name:	Triacylglycerol lipase
Common names:	Lipase; triglyceride lipase; tributyrase; butyrinase; glycerol ester hydrolase; tributyrinase; Tween hydrolase; steapsin; triacetinase; tributyrin esterase; Tweenase; amno N-AP; Takedo 1969-4-9; Meito MY 30; Tweenesterase;

[1] International Union of Biochemistry and Molecular Biology.

IUBMB enzyme nomenclature:	EC 3.1.1.3
Host microorganism:	*Trichoderma reesei*
CAS[2] registry number:	9001-62-1
Reaction:	Triacylglycerol + H_2O = diacylglycerol + carboxylate

2.1.2 Purity and specifications

There are international specifications for enzyme preparations used in the production of food established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in its Compendium of Food Additive Specifications (JECFA 2016) and in the Food Chemicals Codex (Food Chemicals Codex 2014). Section S3—2 of Schedule 3 Identity and Purity, of the Code lists these as primary sources of specifications. Enzyme preparations must meet these specifications. Schedule 3 of the Code also includes specifications for heavy metals in section S3—4.

Table 2 provides a comparison of the product specifications with the international specifications established by JECFA as well as those detailed in the Code (as applicable).

Triacylglycerol lipase from GM *T. reesei* containing a triacylglycerol lipase gene from *T. lanuginosusas* meets international and Code specifications.

Table 2: Product specifications for commercial enzyme preparation compared to JECFA and Code specifications for enzymes.

Analysis	Specifications		
	Product	**JECFA**	***The Code (heavy metals)***
Lead (mg/kg)	<0.05	≤5	≤2
Arsenic (mg/kg)	<0.5		≤1
Mercury (mg/kg)	<0.05		≤1
Cadmium (mg/kg)	<0.03		≤1
Antimicrobial activity	Not detected	Not detected	
Coliforms (cfu/g)	≤30	≤30	
Salmonella (/25 g)	Not detected	Absent	
E. coli (/25g)	Not detected	Absent	
Production strain	Not detected		

[2] Chemical Abstracts Service

2.2 Manufacturing process

The fermentation process of microbial food enzymes is similar across the world. This is also true for the recovery process, in most cases the enzyme is partially separated from the other organic material present in the food enzyme (Raveendran *et al.* 2018, Sharma *et al.* 2020).

The triacylglycerol lipase enzyme is produced by submerged fermentation of the genetically modified strain of *T. reesei.* Once fermentation is complete, the broth containing the enzyme undergoes separation and concentration to produce the final commercial enzyme preparation. The food enzyme prosecution process follows current Good Manufacturing Practices (GMP) and the principles of Hazard Analysis of Critical Control Points (HACCP).

2.3 Technological function and justification

The food industry uses triacylglycerol lipases for various purposes, such as in the production of baked goods, dairy products, and oils. It is employed to hydrolyse fats into simpler compounds, which can influence the texture, flavour, and shelf life of food products (Ferreira-Dias *et al.* 2013, Bárcenas *et al.* 2003).

The enzyme of interest would be used as a processing aid in baking and other cereal-based processes. Triacylglycerol lipase catalyses the hydrolysis of ester bonds of triacylglycerols (or triglycerides), resulting in the formation of mono - and diacylglycerols, free fatty acids, and glycerol. This assists with gas retention and volume of baked products, enhances the dough structure and behaviour during baking and inhibits the swelling of starch and leakage of amylose during cooking.

Use of commercial enzyme preparations follow Good Manufacturing Practice (GMP), i.e., use at a level necessary for the desired enzymatic reaction. The technical information sheet provided with the application provides advice for the optimum use level.

The technological justification for using triacylglycerol lipase is that it breaks down triglycerides to produce benefits (Damodaran *et al*., 2008, Ferreira-Dias *et al*. 2013) including facilitating handling of dough, improving stability, strength, structure, performance, and emulsification and regulating batter viscosity, specifically in producing waffles, pancakes, and biscuits (Enas *et al*. 2003, Fernandes 2010, Gulia *et al.*, 2013).

2.4 Potential presence of allergens

Wheat flour containing gluten is a constituent of the commercial enzyme preparation. If this is the case, then the requirements of paragraph 1.2.3—4(1)(b)(i) relating to mandatory declaration of certain foods or substances in food relating to cereals containing gluten is relevant to any food produced using the enzyme preparation.

2.5 Food Technology conclusion

The stated purpose of this enzyme preparation for use as a processing aid is clearly articulated in the application.

The evidence supports the proposed uses and provides assurance that using the enzyme in the form and prescribed amounts is technologically justified, effective in achieving its stated purpose whilst meeting purity specifications.

Mandatory declaration relating to cereals containing gluten is relevant to any food produced using the enzyme preparation.

3 Safety Assessment

3.1 History of use of the organisms

3.1.1 Host organism

T. reesei (previously known as *Trichoderma viride*) is a common, hypercellulolytic, soil fungus that was initially isolated from deteriorating canvas made from cellulosic material. Strain QM6a is the wild type of practically all *T. reesei* industrial production strains including the strain relevant to this application, AR-822 (Nevalainenet et al., 1994). *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 (Environment Protection Authority), 2020).

T. reesei has a long history of safe use in industrial-scale enzyme production (Nevalainen et al. 1994; Blumenthal 2004; Frisvad et al. 2018). Enzyme preparations from this species are used in food, animal feed, pharmaceutical, textile, detergent, bioethanol and pulp and paper industries. Food enzymes deriving from *T. reesei* strains (including recombinant *T. reesei* strains) have been evaluated by JECFA and many countries which regulate the use of food enzymes, such as Australia, USA, France, Denmark and Canada, resulting in the approval of the use of food enzymes from *T. reesei* in the production of various foods, such as baking, brewing, juice production, wine production and the production dairy products.

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; Nevalainen and Peterson, 2014; Gryshyna et al., 2016). *T. reesei* QM6a strains are known to produce the peptaibol antibiotic paracelsin, but industry-standard submerged fermentation conditions are not linked to the production of paracelsin (US EPA, 2020).

FSANZ has previously assessed the safety of *T. reesei* as the production organism for several food processing aids. Within the Code, Schedule 18 to Standard 1.3.3 currently permits the following enzymes derived from T. reesei: α-Amylase, α-Arabinofuranosidase, α-Glucosidase, β-Glucanase, Aspergillopepsin I, Chymosin, Cellulase, Endo-1,4-beta-xylanase, Glucoamylase, Glucose oxidase, Hemicellulase multicomponent enzyme, Lysophospholipase, Polygalacturonase or Pectinase multicomponent enzyme, Thermomycolin, and Triacylglycerol Lipase. Triacylglycerol lipase enzyme produced *by T. reesei* has been previously approved twice by FSANZ.

The production strain used to produce the lipase enzyme is *T. reesei* strain AR-822. This species identification was confirmed by FSANZ with CCI genomic data provided by the applicant.

The stability of the production strain was demonstrated phenotypically through consistent batch parameters and application of suitable microbiological controls through production. Additionally, the organism was not detected within the final enzyme product in three

independent fermentation batches. The data provided by the applicant also demonstrated that this fungal strain does not produce toxicologically significant amounts of mycotoxins. No public health and safety concerns were identified, and the production organism has been determined to be neither pathogenic nor toxigenic.

3.1.2 Gene donor organism

The *T. reesei* production strain A-822 is genetically modified with the triacylglycerol lipase gene from *Thermomyces lanuginosus*. *T. lanuginosus* was formerly known as *Humicola lanuginose* and has been previously assessed by FSANZ. The donor organism's identity was confirmed via NCBI blast method using the enzyme's amino acid sequence provided by the applicant.

3.2 Characterisation of the genetic modifications

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

A protein engineered variant of the triacylglycerol lipase gene was introduced into the genome of the host *T. reesei,* producing the production strain. The triacylglycerol lipase gene was placed under the control of a *T. reesei* promoter. Data provided by AB Enzymes and analysed by FSANZ confirmed the identity of the protein engineered triacylglycerol lipase enzyme.

An expression cassette containing the triacylglycerol lipase gene was integrated at specific integration sites in the host's genome. The inserted expression cassette also contained the *amdS* selectable marker gene from *Aspergillus nidulans* (Hynes *et al*. 1983; Kelly and Hynes, 1985). The final production strain was selected based on growth on media containing acetamide and high triacylglycerol lipase activity.

The expression cassette was integrated into the genome of the host strain through standard transformation techniques using methodologies described in Penttilä *et al*. (1987) with modifications. While a plasmid was used in the construction of the expression cassette, no plasmid derived sequences were integrated.

3.2.2 Characterisation of the inserted DNA

Whole genome sequencing (WGS) data provided by AB Enzymes confirmed the presence of the inserted DNA in the production strain. The applicant also provided the results of Southern blot analysis which confirmed the absence of vector DNA in the production strain.

3.2.3 Stability of the introduced DNA

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

To provide further evidence of the stability of the introduced phospholipase gene, the applicant provided results of Southern blot analysis and phenotypic data from three fermentation batches of the production strain. This data confirmed that the triacylglycerol lipase gene is expressed over multiple generations and is stable.

3.3 Safety of triacylglycerol lipase

3.3.1 History of safe use

Triacylglycerol lipase is currently permitted as a processing aid in Schedule 18 of the Code. However, triacylglycerol lipase from *T. reesei* containing the gene for triacylglycerol lipase

from *T. lanuginosus* is not permitted and does not have a history of safe use in Australia or New Zealand.

Trichoderma reesei is an approved host microorganism and production strain for several enzymes in Schedule 18 and around the world, with a long history of safe use (Blumenthal 2004; Frisvad *et al*. 2018; Nevalainen *et al*. 1994; Olempska-Beer *et al*. 2006).

Triacylglycerol lipase preparations from various microbial sources are used as processing aids in a range of countries, including Australia and New Zealand. FSANZ has approved the use of triacylglycerol lipase as a processing aid, from both animal origin (bovine, ovine and porcine) and fungal sources, with the use of this enzyme as a processing aid in food spanning more than 30 years.

The applicant has indicated that dossiers on the triacylglycerol lipase enzyme have been submitted in Brazil, the EU and USA, with plans to also make a submission in Canada.

There are no known reports of adverse effects arising from the consumption of *Trichoderma reesei* food enzymes, nor lipases from other production organisms, when used as processing aids.

3.3.2 Bioinformatic assessment of enzyme toxicity

The applicant performed a search for homology of the triacylglycerol lipase enzyme amino acid sequence to known toxins in the NCBI Identical Protein Groups (IPG) database using BLAST-P. The search was performed in June 2023. No significant homologies were found.

3.3.3 Evaluation of toxicity studies

The applicant submitted toxicity studies performed with an enzyme (CCI) produced by a *T. reesei* strain (AR-852) in the same safe strain lineage as the production strain for triacylglycerol lipase (AR-882). *T. reesei* AR-822 is derived from the same parental strain and host strain as *T. reesei* AR-852. This safe strain lineage concept is consistent with Food and Agriculture Organization/World Health Organization guidance on risk assessment of food enzymes (FAO/WHO 2020).

The expression constructs are similar, only differing by the expression cassette for the enzyme gene of interest. These expression cassettes are similar and stably integrated into the genome of the strains without any additional growth/mutagenesis cycles thereafter. The manufacturing conditions for the two production strains are also similar, with only minor changes in fermentation medium that is consistent with common industry practice.

The applicant also assessed the safety of the *T. reesei* AR-822 production strain according to the decision tree of Pariza and Johnson (2001). Pariza and Johnson (2001) and Pariza and Cook (2010) have published guidelines for the safety assessment of microbial enzyme preparations. The safety assessment of a given enzyme preparation is based upon an evaluation of the toxigenic potential of the production organism. The applicant followed a decision tree pathway with the outcome that the *T. reesei* AR-822 production strain is accepted as safe for its intended use (due to demonstrated safe strain lineage of the production strain).

The *T. reesei* AR-852 enzyme preparation was the test item used in the toxicity studies and this is considered suitably equivalent for assessing the safety of *T. reesei* AR-882 triacylglycerol lipase.

3.3.3.1 Animal studies

90-day Repeat-dose oral toxicity study in rats ([Redacted], 2020). Regulatory Status: GLP; conducted according to OECD (Organisation for Economic Cooperation and Development)

TG 408 (2018), Commission Regulation (EC) No. 440/2008 L 142 (2008), and Commission Directive 2001/59/EC (2001).

A *Trichoderma reesei* enzyme preparation was administered daily by oral gavage to Wistar (Crl: WI(Han)) rats (10/sex/group) at doses of 0, 100, 300, or 1000 mg TOS/kg bw/day, for 90 days. The vehicle/negative control was sterile water, and the dose volume was 5 mL/kg bw/day. The animals were group housed (5/sex/group/cage) under standard laboratory conditions of environment and husbandry.

Animals were observed daily for clinical condition and behavioural changes, and twice daily for morbidity and mortality. Detailed clinical observations, body weights and feed consumption were recorded weekly. A functional battery of tests was performed on each animal in the last 2 weeks of dosing, including sensory reactivity, grip strength, and motor activity assessments. Ophthalmic examination was conducted on all animals in the last week of the treatment period. Prior to necropsy, blood and urine samples were collected for haematology, clinical chemistry, and urinalysis. All animals underwent a detailed necropsy at study termination, including full macroscopic examination of an extensive range of organs and tissues. Histopathological examination was conducted on selected organs and tissues from the control and high-dose group animals.

All animals survived to the end of the treatment period. No treatment-related effects were observed on clinical signs, feed/water consumption, body weights, ophthalmology, functional observations, or urinalysis. Some minor changes in haematology and clinical chemistry parameters were observed following the treatment period in males and females from all dose groups. However, these were not considered to be treatment-related because the changes were within the range of historical reference data, were not dose dependent, and only occurred in one gender.

At necropsy, some incidental findings in individual animals from all treatment groups were observed macroscopically, however no changes were observed upon histopathological examination. No other treatment-related macroscopic or histopathological findings were observed at necropsy.

It was concluded that the no observed adverse effect level (NOAEL) of the enzyme preparation was 1000 mg TOS/kg bw/day, the highest dose tested, under the conditions of this study.

3.4.3.2 Genotoxicity studies

Reverse Mutation Assay using Bacteria ([Redacted], 2018). Regulatory Status: GLP; conducted according to OECD TG 471 (1997), Commission Regulation (EC) No. 440/2008 B.13/14 (2008), and EPA OPPTS 870.5100 (1998).

The potential mutagenicity of a *Trichoderma reesei* enzyme preparation was evaluated in *Salmonella enterica* ser. Typhimurium strains TA98, TA100, TA1535, TA1537 and TA102, with and without metabolic activation using rat liver homogenate (S9 mix). Appropriate positive control articles, as recommended by the OECD guideline, were used. Sterile water was used as the vehicle/negative control.

Two experiments were conducted: using the plate incorporation method (experiment I) and the pre-incubation method (experiment II). All tests were conducted in triplicate and the following concentrations of test article were tested: 31.6, 100, 316, 1000, 2500 and 5000 µg/plate. No precipitation or toxicity was observed at any concentration of the test article.

No biologically relevant increases in revertant colony numbers of any of the five tester strains were observed at any concentration level (up to and including 5000 µg TOS/plate), with or without S9 mix, in both experiments I and II. The expected increases in revertant colony numbers were observed with all the positive control articles used, therefore confirming the validity of the assay.

It was concluded that the test article showed no evidence of mutagenic activity under the conditions of the assay.

In vitro Mammalian Micronucleus Assay in Human Lymphocytes ([Redacted], 2019). Regulatory Status: GLP; conducted according to OECD TG 487 (2016) and Commission Regulation (EU) 2017/735 (2017).

The potential of a *Trichoderma reesei* enzyme preparation to induce micronuclei formation in mammalian cells was tested using human lymphocytes isolated from the peripheral blood of one donor (of unspecified age and sex).

Test article concentrations (based on a dose-range finding experiment) were 250, 500 and 2500 µg/mL without S9 mix and 500, 1000, 2000 and 4000 µg/mL with S9 mix in the 4-hour exposure protocol (Experiment I); and 125, 175 and 200 µg/mL without S9 mix in the 44-hour exposure protocol (Experiment II). Appropriate positive control articles, as recommended by the Guideline, and negative control (culture medium) were also assayed.

No precipitation of the test article was observed at any concentration, but some evidence of cytotoxicity was observed. In Experiment I, increased cytostasis was observed at 2500 µg/mL without S9 and at $\geq$1000 µg/mL with S9. In Experiment II an increase in cytostasis was observed at 200 µg/mL.

No biologically relevant increase in the micronucleus frequency was noted after treatment with the test article in either experiment, with or without S9 mix for metabolic activation. The positive control articles induced the expected statistically significant increases in the frequencies of micronuclei, confirming the validity of the assay.

It was concluded that the enzyme preparation produced by *Trichoderma reesei* did not induce structural and/or numerical chromosomal damage in human lymphocytes and is non-mutagenic in the *in vitro* mammalian cell micronucleus assay.

3.3.4 Potential for allergenicity

Searches for homology of the lipase enzyme amino acid sequence with those of known allergens were performed in February 2023 by the applicant using the AllergenOnline database[3]. The following searches were conducted:

- Alignment (FASTA) of the entire query amino acid sequence to known allergens (more than 35% identity)
- Alignment (FASTA) of sliding 80-amino acid windows of the query protein to known protein allergens (more than 35% identity)
- A search for eight amino acid exact matches.

One match of greater than 35% identity (but below 50% identity) was found using the full-length search. This match had a high E-value indicating a non-significant match (CCI information). It is unlikely that the lipase enzyme is allergenic. Furthermore, EFSA regard the 35% identity cut-off as conservative (EFSA 2010). No matches of greater than 35% identity were found using the 80-mer sliding window search; and no exact matches of eight amino acids were found.

The triacylglycerol lipase enzyme is not expected to pose a food allergenicity concern.

3.3.5 Safety assessments by overseas agencies

Safety assessments of the triacylglycerol lipase enzyme preparation by international agencies or other national government agencies are not available.

The applicant provided a letter confirming that Denmark has approved the use of the enzyme that is the subject of this application. However, this is not a safety assessment and is not accepted by FSANZ as an assessment by an international agency. The applicant advised that dossiers have been submitted to Brazil, the EU, and the USA for approval.

3.3.6 Toxicology conclusions

Triacylglycerol lipase was assessed according to the safe strain lineage concept. The applicant provided commercial-in-confidence information to support the safe strain lineage of the production strain *T. reesei*. A *T. reesei* enzyme preparation showed no evidence of genotoxicity in a bacterial reverse mutation assay or a micronucleus assay in human lymphocytes. In a 90-day oral gavage study in rats, the no-observed-adverse-effect-level (NOAEL) was the highest dose evaluated, 1000 mg total organic solids (TOS) /kg bw/day. Bioinformatic data indicate a lack of homology with known toxins or allergens, and the enzyme is unlikely to pose an allergenicity concern. The Theoretical Maximum Daily Intake (TMDI) of the TOS from the triacylglycerol lipase preparation was calculated to be 0.38 mg TOS/kg body weight/day. A comparison of the NOAEL and the TMDI results in a margin of exposure (MOE) of approximately 2,600. FSANZ concludes than an ADI of “not specified” is appropriate for this enzyme.

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

3.4 Dietary Exposure

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

The budget method is a valid screening tool for estimating the 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 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 2020a). The method is used by international regulatory bodies and the FAO/WHO Joint Expert Committee on Food Additives (JECFA) (FAO/WHO 2021) for dietary exposure assessments for processing aids.

Triacylglycerol lipase enzyme from the same source is permitted for use as a processing aid to produce bakery products and cereal-based beverages. In the application assessed under A1159 the maximum use level of 21.2 mg TOS/kg raw material (flour) was used in the budget method calculation for solid food. As FSANZ did not review the budget method calculation submitted in the application assessed under A1159 and this level is higher than the maximum use level of 4 mg TOS/kg in the raw material (cereals) in the current application, FSANZ has used the higher concentration in its worst-case scenario approach.

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

- the maximum physiological requirement for solid food (including milk) is 25 g/kg body weight/day
- Fifty percent of solid food is processed
- all solid foods contain the maximum use level of 4 mg TOS/kg in the raw material (cereals)
- a maximum ratio of 0.71 for raw material (cereals) weight to final food (baked products) weight
- the maximum physiological requirement for liquid is 100 mL/kg body weight/day (the standard level used in a budget method calculation for non-milk beverages)
- Twenty-five percent of non-milk beverages are soft drinks
- the enzyme preparation is not added to any non-milk beverages
- all the TOS from the enzyme preparation remains in the final food
- all producers use this triacylglycerol lipase preparation at the highest use level
- the final foods containing the theoretical amount of the triacylglycerol lipase preparation would be consumed daily over the course of a lifetime.

Based on these assumptions, the applicant calculated the TMDI of the TOS from the enzyme preparation to be 0.036 mg TOS/kg body weight/day.

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

- The maximum physiological requirement for solid food (including milk) is 50 g/kg body weight/day (the standard level used in a budget method calculation where there is

potential for the enzyme preparation to be in baby foods or general-purpose foods that would be consumed by infants).

- FSANZ would assume 12.5% of solid foods contain the enzyme based on commonly used default proportions noted in the FAO/WHO Environmental Health Criteria (EHC) 240 Chapter 6 on dietary exposure assessment (FAO/WHO 2009). However, the applicant has assumed a higher proportion of 50% based on the nature and extent of use of the enzyme and therefore FSANZ has also used this proportion for solid foods as a worst-case scenario.
- The concentration of triacylglycerol lipase in solid final foods will not exceed the maximum level of 21.2 mg TOS/kg raw material (flour) (the level used in the application for A1159).

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 was 0.38 mg TOS/kg body weight/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 the assumption that all the TOS from the enzyme preparation remains in the final foods and beverages whereas the applicant has stated that the enzyme is likely to either be inactivated or removed during processing. If any inactivated enzyme remained after processing, it would be present in insignificant quantities and perform no function in the final food.

4 Conclusion

The proposed use of this triacylglycerol lipase as an enzyme processing aid in the quantity and form proposed to be used, is consistent with its typical function of hydrolysing lipids. Triacylglycerol lipase performs its technological purpose in the food for sale, therefore functioning as a processing aid for the purposes of the Code. The enzyme meets relevant identity and purity specifications.

There are no safety concerns from the use of triacylglycerol lipase from GM *T. reesei.* Triacylglycerol lipases from other sources have a long history of safe use in food. The production organism is neither pathogenic nor toxigenic. Analysis of the GM production strain confirmed the presence and stability of the inserted DNA.

The TMDI of the TOS from the triacylglycerol lipase preparation is 0.38 mg TOS/kg bw. A comparison of the NOAEL and the TMDI results in an MOE of approximately 2,600.

Based on the reviewed data, in the absence of any identifiable hazard, ADI 'not specified' is appropriate

Overall, FSANZ concludes that there are no safety concerns from the use of protein engineered triacylglycerol lipase from a genetically modified strain of *T. reesei* containing an encoding gene from *T. lanuginosus.*

5 References

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