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

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Supporting document

Risk and technical assessment – Application A1221

Phospholipase A1 from GM *Aspergillus niger* as a processing aid

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 phospholipase A1 (EC 3.1.1.32) as a processing aid for use in the manufacture of edible vegetable oils and fats products. This enzyme is sourced from genetically modified (GM) *Aspergillus niger,* containing the phospholipase A1 gene from *Evansstolkia leycettana* (basionym *Talaromyces leycettanus*).

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 public health and safety concerns that may arise from the use of this enzyme.

FSANZ concludes that the proposed use of the enzyme in the processing of vegetable oils is consistent with its typical function of catalysing the hydrolysis of phospholipids. This converts the phosphatides in vegetable oil to a hydrated gum that can easily be removed before further refining. This process is known as 'degumming'.

Analysis of the evidence provides adequate assurance that the proposed use of the enzyme, in the requested amount (a level not higher than necessary to achieve the desired enzyme reaction under good manufacturing practice (GMP)), is technologically justified.

Phospholipase A1 performs its technological purpose during the production of food and is not performing a technological purpose in the final food. It is therefore appropriately categorised as a processing aid as defined in the Code.

There are relevant identity and purity specifications for the enzyme in the Code and the applicant provided evidence that the enzyme meets these specifications.

No public health and safety concerns were identified in the assessment of phospholipase A1 from GM *A. niger* under the proposed use conditions. *A. niger* has a long history of safe use as a source of enzyme processing aids, including many that are already permitted in the Code. The *A. niger* host is neither pathogenic nor toxigenic.

Analysis of the modified production strain confirmed the presence and stability of the inserted DNA.

The enzyme does not show any appreciable sequence homology with known toxins. No treatment-related adverse effects were observed in a 90-day oral gavage study in rats. It was concluded that the no observed adverse effect level (NOAEL) was the highest dose tested, 1356 mg total organic solids (TOS)/kg bw/day. The enzyme was not genotoxic *in vitro*. Results of a sequence homology assessment found no significant homology with known allergens and the enzyme is not anticipated to pose any allergenic concern in food.

The theoretical maximum daily intake (TMDI) based on FSANZ's calculations for solid food and non-milk beverages is 0.006 mg TOS/kg body weight/day. Comparison of the NOAEL and the TMDI results in a margin of exposure (MOE) of around 226,000.

In the absence of any identifiable hazard an acceptable daily intake (ADI) 'not specified' is appropriate for phospholipase A1 from *E. leycettana*, expressed in GM *A. niger*.

Table of contents

1 Introduction

Novozymes Australia Pty Limited applied to Food Standards Australia New Zealand (FSANZ) to permit the use of the enzyme phospholipase A1 (EC 3.1.1.32) as a processing aid in the manufacture of edible vegetable oils and fats products. Following subsequent consultation with Food Standards Australia New Zealand (FSANZ) however, the applicant has clarified that the technological purpose of this enzyme is as a processing aid in the processing of vegetable oils, specifically the degumming (removal of phosphatides) of those oils, which can then be used in the manufacture of fat-based products. The focus of FSANZ's assessment was therefore on the use of the enzyme in the processing of vegetable oils.

This enzyme is sourced from a genetically modified (GM) strain of *Aspergillus niger,* containing the phospholipase A1 gene from *Evansstolkia leycettana* (basionym *Talaromyces leycettanus*). The gene donor host named in the application is *Talaromyces leycettanus. Talaromyces leycettanus* is now however, named as *Evansstolkia leycettana* (see Section 3.1.2).

1.1 Objectives of the assessment

Currently, Schedule 18 of the Australia New Zealand Food Standards Code (the Code) includes permission for phospholipase A1 from GM *Aspergillus oryzae*. This phospholipase A1 produced by GM *A. niger* requires a pre-market assessment before permission can be given for its use as a processing aid.

The objectives of this risk and technical assessment were to:

- determine whether the proposed purpose is a solely technological purpose (function) and that the enzyme achieves its technological purpose as a processing aid in the quantity and form proposed to be used
- evaluate potential public health and safety 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), and
  - safety of the enzyme.

2 Food technology assessment

2.1 Characterisation of the enzyme

The production microorganism of the enzyme is a GM strain of *A. niger*. The applicant provided relevant information regarding the identity of the phospholipase A1 enzyme. FSANZ verified this using the International Union of Biochemistry and Molecular Biology (IUBMB) enzyme nomenclature database (McDonald et al 2009). Details of the identity of the enzyme are provided in Table 1.

Table 1 *Identity*

Generic common name:	Phospholipase A1
Accepted IUBMB name:	Phospholipase A_1
Systematic name:	Phosphatidylcholine 1-acylhydrolase

Other names:	PS-PLA1 Phosphatidylserine-specific phospholipase A_1
EC number:	3.1.1.32
Reaction:	phosphatidylcholine + H_2O = 2-acylglycerophosphocholine + a carboxylate

For a graphical representation of the hydrolysis reaction catalysed by phospholipase A1, refer to its record in the enzyme database BRENDA (Chang et al 2021).

2.2 Manufacturing process

2.2.1 Production of the enzyme

Novozymes' phospholipase A1 is produced by submerged fermentation of the GM strain of *A. niger*. The fermentation steps are inoculum, seed fermentation, and main fermentation. A recovery stage follows fermentation, involving primary and liquid separation, germ filtration, concentration to achieve the desired enzyme activity, evaporation and stabilisation to provide a concentrated enzyme solution free of the production strain and insoluble substances. This is followed by formulation of the enzyme into an enzyme preparation. 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. Novozymes' phospholipase A1 enzyme preparation is sold as a liquid product consisting of glycerol, potassium sorbate, sodium benzoate, and sorbitol. The production is manufactured in accordance with current Good Manufacturing Practice for Food (cGMP) and the principles of Hazard Analysis and Critical Control Point (HACCP).

The application states that all raw materials used in the fermentation and recovery processes are standard ingredients of food grade quality that meet predefined quality standards. The raw materials conform to either specifications set out in the Food Chemicals Codex, 12th edition, 2020 or regulations applying in the European Union.

Details on the manufacturing process, raw materials and ingredients used in the production of the phospholipase A1 enzyme preparation were provided in the application or as Confidential Commercial Information (CCI).

2.2.2 Allergen considerations

The Product Data Sheet for the enzyme preparation states that the following allergens are not present: cereals containing gluten, crustaceans, egg, fish, lupin, milk (including lactose), molluscs, nuts, peanuts, sesame, soy, sulphites. The applicant provided additional information as CCI, supporting the absence of these allergens in their enzyme preparation and in vegetable oils manufactured with 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 meets the requirements in both of these 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 a representative batch of the phospholipase A1 preparation. Table 2 provides a comparison of the analyses with international specifications established by JECFA and Food Chemicals Codex, as well as those in the Code (as applicable). Based on these results, the enzyme preparation met all relevant specifications for arsenic and metals and the microbiological criteria.

Table 2 *Comparison of Novozymes phospholipase A1 preparation compared to JECFA, Food Chemicals Codex, and Code specifications for enzymes*

		Specifications		
Analysis	**Analysis provided by manufacturer**	***JECFA (2006)***	***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
Coliforms (CFU/g)	<4	≤30	≤30	-
***Salmonella* (in 25 g)**	Not detected	Absent	Negative	-
***E. coli* (in 25 g)**	Not detected	Absent	-	-
Antibiotic activity	Not detected	Absent	-	-

Whilst the manufacturing processes ensure the production microorganism is removed 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 3.4 below for the total organic solids (TOS) value.

2.3 Technological purpose of the enzyme

Novozymes' phospholipase A1 is intended for use as a processing aid in the degumming of vegetable oils.

Vegetable oils can be produced from parts of many different plants, including sunflower, canola, peanut, and coconut. After extraction, all contain a variety of unwanted components that affect taste, appearance, and quality. Before these impurities can be removed by various refining techniques, the oil must be degummed to remove phosphatides, which affect storage stability.

Phospholipase A1 belongs to the phospholipase class of enzymes, specifically the acyl hydrolases. Acyl hydrolases catalyse hydrolysis, esterification, and transesterification in phospholipids to remove and replace the acyl chain. Specifically, phospholipase A1 hydrolyses ester bonds in the sn-1 position of phospholipids including phosphatides. This releases lysophospholipids, such as lysophosphatides, and free fatty acids. The lysophosphatides released form a hydrated gum, which is not soluble in oil. The gum can subsequently be removed by physical separation methods. This process is known as 'degumming'.

The stated technological purpose of the phospholipase A1 enzyme is supported by scientific literature (e.g. Casado et al 2012, Lamas et al 2014).

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

Table 3 *Physical and chemical properties of Novozymes' phospholipase A1 enzyme preparation*

Physical and chemical properties of commercial enzyme preparation	
Enzyme activity	75 PLA-L/g
Appearance	Colourless to yellow liquid
Recommended storage temperature range	0–25°C
Density	1.17 g/mL

PLA-L/g: phospholipase A1 units per gram

The enzyme preparation is available as a liquid concentrate, standardised in phospholipase A1 units (PLA-L) to 75 PLA-L/g. The application includes a description of Novozymes' method used to determine phospholipase A1 activity. In summary, phospholipase A1 is used to hydrolyse the ester bond in para-nitrophenyl (pNP)-palmitate. The pNP released can be detected using spectrophotometry. The increase in absorbance at 340 nm correlates to the amount of product formed, which is proportional to the enzyme activity. The highest level used in food manufacturing is 5 PLA-L per kilogram of oil.

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 requested use of the enzyme at GMP levels.

2.4 Technological justification

As outlined above, the technological purpose of phospholipase A1 is the conversion of phosphatides in vegetable oil to a hydrated gum that can be easily removed before further refining. Removing phosphatides at this stage of processing improves the stability of the oil.

The enzyme performs its function of catalysing the hydrolysis of ester bonds in phospholipids during the processing of vegetable oils. It is therefore performing as a processing aid for the purposes of the Code.

The Code already permits phospholipase A1, from a different source (GM) source, to be used in the manufacture of all foods. The specific benefits of the action of phospholipase A1 in the processing of vegetable oils are described below.

Vegetable oils can be degummed by a variety of techniques. However, enzymatic degumming using phospholipases has several advantages over acid degumming and water degumming. Crude vegetable oils contain high levels of phosphorus in the form of phospholipids, which must be reduced before further processing to limit degradation of the oil. Enzymatic processes are more effective than water or acid techniques at removing phospholipids, especially those with non-hydratable phosphatides. Phospholipase A1 has lower specificity than phospholipase A_2 and therefore supports a higher removal of phosphorus (IUBMB 2022).

2.5 Food technology conclusion

FSANZ concludes that the proposed use of this phospholipase A1 as an enzyme in the processing of vegetable oils is consistent with its typical function of catalysing the hydrolysis of phospholipids. This converts the phosphatides in vegetable oil to a hydrated gum that can easily be removed before further refining of the oil. FSANZ concludes that the evidence presented to support the proposed use provides adequate assurance that the use of the enzyme, in the form and requested amount (a level not higher than necessary to achieve the desired enzyme reaction, according to GMP levels), is technologically justified and has been demonstrated to be effective in achieving the stated purpose.

Phospholipase A1 performs its technological purpose during the production of food and is not performing a technological purpose in the final food. It is therefore appropriately categorised as a processing aid for the purposes of the Code.

There are relevant identity and purity specifications for the enzyme in the Code and the applicant provided evidence that their enzyme meets these specifications.

3 Safety assessment

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

A. niger is widely used as a production organism and host for the manufacture of food ingredients and enzymes. *A*. *niger* is recognised as neither pathogenic nor toxigenic. The applicant has used the *A*. *niger* BO-1 strain lineage for food enzyme production for over 30 years. FSANZ has previously assessed a host organism from the BO-1 strain lineage in an application for glucan 1,4-alpha-glucosidase (EC 3.2.1.3) (A1184). The identity of the host organism was determined using standard molecular techniques.

3.1.2 Gene donor organisms

The gene donor for phospholipase A1 gene is identified in the application as *Talaromyces leycettanus*. A recent paper by Houbraken et al. (2020) reviewed the family of *Aspergillaceae* fungi using phylogenetic methods. An outcome of the review was the creation of new genera including *Evansstolkia* and new combination species *Evansstolkia leycettana* (H.C. Evans & Stolk) Houbraken, Frisvad & Samson comb. nov.

GenBank lists the legitimate name as *Evansstolkia leycettana* (H.C. Evans & Stolk) Houbraken, Frisvad & Samson, Studies in Mycology 95: 89 (2020) [MB#832558] with a basionym *Talaromyces leycettanus* H.C. Evans & Stolk, Transactions of the British Mycological Society 56: 45 (1971) [MB#324419]. Therefore, the name of the gene donor organism in this application is considered to be *Evansstolkia leycettana* (basionym *Talaromyces leycettanus*) and is referred to in this report as *E. leycettana*.[1]

[1] FSANZ notified the applicant about the name change and they have accepted *Evansstolkia leycettana* (basionym *Talaromyces leycettanus*) as the name of the donor species. The term 'basionym' means the original name on which the new name is based.

3.2 Characterisation of the genetic modification(s)

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

The gene that encodes the phospholipase A1 gene was synthesised *in vitro* based on the sequence from *E. leycettana* available in public databases. Data provided by Novozymes and analysed by FSANZ confirmed the expected phospholipase A1 amino acid sequence.

The phospholipase A1 gene was introduced into the genome of the host strain, *A. niger* and placed under the control of a hybrid *Aspergillus* promoter, a terminator from *A. niger* and a leader sequence from a native gene from *A. niger*. The *amdS* gene from *A. nidulans* (Corrick et al. 1987) was used as a selective marker enabling the selection of positive transformants by growth on media supplemented with nitrogen (Kelly and Hynes, 1985). The phospholipase A1 gene was integrated at specific integration sites in the host's genome. The final production strain was selected based on growth on medium containing nitrogen and high phospholipase A1 activity.

3.2.2 Characterisation of inserted DNA

Data provided by Novozymes 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 phospholipase A1 gene, the applicant provided phenotypic data from large-scale fermentation of the production strain. These data confirmed that the phospholipase A1 gene is expressed over multiple generations and is stable.

3.3 Safety of the enzyme

3.3.1 History of safe use

The enzyme has been approved for use in Denmark and France since 2018, and Brazil and Mexico since 2019. Confidential sales volume data were provided by the applicant to confirm that it has been sold for commercial use in countries in which specific approval is not required.

3.3.2 Bioinformatics concerning potential for toxicity

A recent (2020) sequence homology assessment of the phospholipase A1 to known toxins was conducted. Amino acid sequences of known protein toxins were extracted from UniProt[2]. Each of the 275426 entries was placed in its own fasta file, and the sequence of the phospholipase A1 was also placed in its own fasta file. The sequence alignment program ClustalW 2.0.10 was used to align each sequence to the phospholipase A1. A summary file containing the length of each sequence and number of identical residues was also created. From this, the identity percentage to the phospholipase A1 sequence or the compared toxin

[2] https:www.uniprot.org

sequence was calculated, whichever was longest. This approach was chosen because the toxin sequences have many different lengths, both much shorter and much longer than that of the phospholipase A1. By always using the longest sequence, artificial high scores from very short or very long toxins are avoided. The largest homology encountered was 19.3%, indicating that the homology to any toxin sequence in this database is random and very low.

3.3.3 Toxicity studies

All the submitted toxicity studies provided were conducted using a batch of the phospholipase A1 enzyme concentrate synthesized in the same way as the commercial product, but without stabilization or standardization for commercial sale.

3.3.3.1 Animal Studies

90-day repeat-dose oral gavage study of phospholipase A1 in Han Wistar rats (Cooper et al 2016; unpublished study). Regulatory status: GLP; in general accordance with OECD test guideline 408

The vehicle and control article for this study was reverse osmosis water, in which the test article was completely miscible. The highest dose used was undiluted enzyme concentrate, 1356 mg TOS/kg bw/day. Low and middle doses were 136 and 447 mg TOS/kg bw/day respectively. Dose formulations were sampled for enzyme concentration in Weeks 1, 6 and 13 of the in-life phase. Rats were received at 42 to 48 days old, and acclimatized for 13 days and subject to ophthalmological examinations before being assigned to groups, 10/sex/group. Rats were group-housed, 5/cage, under standard laboratory conditions of environment and husbandry. Food and water were provided *ad libitum,* except prior to blood collection.

Parameters determined during the study included survival, clinical observations, bodyweight changes, food consumption and water consumption. In Week 12, all rats were assessed for sensory reactivity, grip strength and motor activity, and ophthalmological examinations were performed on control and 1356 mg TOS/kg bw/day (high dose) rats. In Week 13, rats were anaesthetised for collection of blood for haematology, measurement of coagulation times, and clinical chemistry. Rats were then killed, and detailed necropsy was performed. Fresh organ weights, as sex-appropriate, were recorded of adrenals, brain, epididymides, heart, kidneys, liver, ovaries, spleen, testes, thymus, and uterus. A comprehensive list of organs and tissues was preserved for histopathology.

All rats survived to the end of the in-life phase and there were no treatment-related effects on any of the parameters measured, with the exception of liver weight. The group mean value for absolute liver weight of the 1356 mg TOS/kg bw/day males was slightly increased (7.8%) relative to that of male controls, but there was no associated histopathology, and serum markers of liver damage were not elevated. It was concluded that the no observed adverse effect level (NOAEL) was the highest dose tested, 1356 mg TOS/kg bw/day.

3.3.3.2 Genotoxicity

Bacterial reverse mutation assay of beta-amylase (Ballantyne et al 2016; unpublished study). Regulatory status: GLP; in general accordance with OECD test guideline 471

The bacterial reverse mutation assay was conducted under GLP conditions and in general accordance with OECD guideline 471, although the exposure in liquid culture (the “treat and plate” method) used is not described in any Guideline. This method was used because an enzyme concentrate is likely to contain free histidine and tryptophan, which could cause a “feeder effect” on bacterial colony growth, and result in false positive results. Bacterial test strains used in the assay were *Salmonella enterica* ser. Typhimurium strains TA1535, TA100, TA1537, and TA98 and *Escherichia coli* WP2uvrApKM101. The solvent and negative control article was deionised water. Bacteria were exposed to phospholipase A1 in a phosphate-buffered nutrient broth for 3 hours, at concentrations of 16, 50, 160, 500, 1600

and 5000 µg TOS/mL in Experiment 1, and over a narrower range of 160, 300, 625, 1250, 2500 and 5000 µg TOS/mL in Experiment 2. The test article was removed by centrifugation prior to plating of the bacteria. Each assay was conducted in triplicate, in the absence and presence of S9 mix for metabolic activation. Tests with appropriate positive control articles were run in parallel.

In Experiment 1, evidence of toxicity of the phospholipase A1 to the bacteria was limited to slight thinning of the bacterial lawn for TA 98, in the presence of S9, only at 5000 µg TOS/mL. There was no clear evidence of toxicity observed in Experiment 2. A very small increase in revertant numbers was observed in strain TA1537, in the absence of S9 in Experiment 1 at all concentration of the enzyme but was not observed in Experiment 2 and was therefore considered unrelated to the test article. The positive control articles induced the expected significant increases in revertant colonies compared to the solvent control plates, confirming the validity of the assay. It was concluded that the enzyme is not mutagenic under the conditions of this assay.

In vitro *micronucleus assay of phospholipase A1 in human peripheral lymphocytes (Whitwell et al 2016; unpublished study). Regulatory status: GLP; in compliance with OECD test guideline 487*

Lymphocytes were harvested from the peripheral blood from two healthy non-smoking female volunteers. The solvent and negative control article was sterile water. Appropriate positive control articles were used; mitomycin C and vinblastine as clastogenic and aneugenic positive control chemicals respectively in the absence of S9 mix, and cyclophosphamide as a clastogenic positive control chemical in the presence of S9 mix. Cultures were exposed to the test substance for three hours in the presence and absence of S9 mix and harvested 24 hours after the beginning of treatment. In addition, a continuous 24-hour treatment without S9 mix was conducted with harvesting 48 hours after the beginning of treatment. Test concentrations of the enzyme were 3000, 4000 and 5000 µg TOS/mL in all experiments. Negative control tests were conducted in quadruplicate while tests of the enzyme concentrate were conducted in duplicate. The cultures were treated with cytochalasin-B after removal of the test substance. One thousand binucleate cells from each culture (2000 per concentration) were analysed for micronuclei.

Treatment of cells with phospholipase A1 in the absence and presence of S9 resulted in frequencies of micronucleated binucleate (MNBN) cells which were similar to and not significantly ($p \leq 0.05$) higher than those observed in concurrent vehicle controls. The positive control articles induced the expected increases in MNBN cells, confirming the validity of the assay. It was concluded that the enzyme did not induce micronuclei in human peripheral blood lymphocytes.

3.3.4 Potential for allergenicity

A recent (2020) sequence homology assessment of the phospholipase A1 enzyme to known allergens was conducted by comparing the amino acid sequence of the enzyme to allergens from the Food Allergy Research and Resource Program (FARRP) allergen protein database[3]. Searches included 35% identity over a sliding window of 80 amino acids, 35% identity over 80 amino acids (scaled), full-length alignment and 100% identity over 8 amino acids. There were no hits with any of the four searches. It is concluded that the phospholipase A1 is not anticipated to pose any allergenic concern in food.

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

3.3.5 Assessments by other regulatory agencies

No safety assessment reports by other regulatory agencies are available. However as noted in section 3.3.1 the enzyme has been approved for use in Denmark and France since 2018, and Brazil and Mexico since 2019.

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 phospholipase A1 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 food additive concentration in foods and beverages, and the proportion of foods and beverages that may contain the food additive. The TMDI can then be compared to an acceptable daily intake (ADI) or a NOAEL to estimate a margin of exposure for risk characterisation purposes. Whilst the budget method was originally developed for use in assessing food additives, it is also appropriate to use for estimating the TMDI for processing aids (FAO/WHO 2020). The method is used by international regulatory bodies and the FAO/WHO Joint Expert Committee on Food Additives (JECFA) (FAO/WHO 2021) for dietary exposure assessments for processing aids.

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

- the maximum physiological requirement for solid food (including milk) is 25 g/kg body weight/day
- 50% of solid food is processed
- all processed solid food contains 25% oils and fats
- the maximum physiological requirement for liquid is 100 mL/kg body weight/day (the standard level used in a budget method calculation)
- 25% of non-milk beverages are processed
- all processed non-milk beverages contain 12% oils and fats
- the density of non-milk beverages is ~1
- all of the enzyme remains in the final food
- all solid foods and non-milk beverages contain the highest use level of 0.67 mg TOS/kg raw material (oils and fats).

Based on these assumptions, the applicant calculated the TMDI of the enzyme to be 0.004 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 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 based on FSANZ's calculations for solid food and non-milk beverages is 0.006 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 that it was assumed that the enzyme remains in the final foods whereas the applicant has stated that it is likely to either be reduced or removed during processing, or would be present in insignificant quantities. In addition the enzyme would be inactivated and perform no function in the final food to which the ingredient is added.

4 Discussion

FSANZ concludes that the proposed use of this phospholipase A1 as an enzyme in the processing of vegetable oils is consistent with its typical function of catalysing the hydrolysis of phospholipids. The evidence presented to support the proposed use provides adequate assurance that the use of the enzyme, in the form and requested amount (i.e. at a level consistent with GMP), is technologically justified and has been demonstrated to be effective in achieving its stated purpose.

Phospholipase A1 performs its technological purpose during the production of food and is not performing a technological purpose in the final food. It is therefore appropriately categorised as a processing aid for the purposes of the Code.

There are relevant identity and purity specifications for the enzyme in the Code and the applicant provided evidence that the enzyme meets these specifications.

No public health and safety concerns were identified in the assessment of phospholipase A1 from GM *A. niger* under the proposed use conditions. *A. niger* has a long history of safe use as a source of enzyme processing aids, including many that are already permitted in the Code. The *A. niger* host is neither pathogenic or toxigenic.

The gene that encodes the phospholipase A1 gene was synthesised *in vitro* based on the sequence from *E. leycettana* available in public databases. Data provided by the applicant and analysed by FSANZ confirmed the expected phospholipase A1 amino acid sequence. The assessment also confirmed the inserted gene is integrated into the genome of the production strain and is stably expressed over multiple generations.

The enzyme has been approved for use in Denmark and France since 2018, and Brazil and Mexico since 2019. Confidential sales volume data were provided by the applicant to confirm that it has been sold for commercial use in countries in which specific approval is not required. The enzyme does not show any appreciable sequence homology with known toxins. No treatment-related adverse effects were observed in a 90-day oral gavage study in rats. It was concluded that the NOAEL was the highest dose tested, 1356 mg TOS/kg bw/day. The enzyme was not genotoxic *in vitro*. Results of a sequence homology assessment found no significant homology to known allergens and the enzyme is not anticipated to pose any allergenic concern in food.

The TMDI based on FSANZ's calculations for solid food and non-milk beverages is 0.006 mg TOS/kg body weight/day. Comparison of the NOAEL and the TMDI results in a margin of exposure (MOE) of around 226,000.

5 Conclusion

In the absence of any identifiable hazard an ADI 'not specified' is appropriate for phospholipase A1 from *E. leycettana*, expressed in GM *A.niger*.

6 References

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