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

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Supporting document 1

Risk and technical assessment – Application A1293

Phosphoinositide Phospholipase C from *Bacillus licheniformis* as a processing aid

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Executive summary

Novozymes Australia Pty Ltd has applied to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of the enzyme phosphoinositide phospholipase C (EC 3.1.4.11), from *Bacillus licheniformis* containing the phosphoinositide phospholipase C gene from *Pseudomonas* sp. 62186, as a processing aid in degumming vegetable fats and oils.

The available evidence provides adequate assurance that the proposed use of phosphoinositide phospholipase C from *B. licheniformis* as a processing aid is technologically justified. Phosphoinositide phospholipase C performs its primary technological function during food processing and, as such, meets the definition of a processing aid for the purposes of the Code. The enzyme preparation meets international purity specifications.

Phosphoinositide phospholipase C from *B. licheniformis* has been approved for use as a processing aid in several countries.

No public health or safety concerns were identified concerning the use of the production organism, which is neither pathogenic nor toxigenic. Analysis of the production strain confirmed the presence and stability of the inserted DNA. No significant homology between the enzyme and any known toxins or allergens was identified. The enzyme preparation is not expected to pose a food allergenicity concern under the proposed conditions of use.

Phosphoinositide phospholipase C showed no evidence of genotoxicity *in vitro*. The no observed adverse effect level (NOAEL) in a 13-week oral toxicity study in rats was 194.5 mg total organic solids (TOS)/kg bw/day.

The theoretical maximum daily intake (TMDI) of the TOS from the enzyme preparation was calculated to be 0.01 mg TOS/kg bw/day. A comparison of the NOAEL and the TMDI results in a Margin of Exposure (MOE) of approximately 19,000.

Based on the large MOE, it is concluded that an acceptable daily intake (ADI) ‘not specified’ is appropriate.

Overall, FSANZ concludes there are no safety concerns from the use of phosphoinositide

phospholipase C from *B*. *licheniformis* in the quantity and form required to perform its typical function in degumming vegetable fats and oils, which must be consistent with Good Manufacturing Practice (GMP).

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1 Introduction

Novozymes Pty Ltd applied to Food Standards Australia New Zealand (FSANZ) to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of the enzyme phosphoinositide phospholipase C (EC 3.1.4.11) from *Bacillus licheniformis* containing the phosphoinositide phospholipase C gene from *Pseudomonas* sp. 62186 as a processing aid in degumming vegetable fats and oils.

The enzyme will be used in the processing of vegetable fats and oils, where it hydrolyses phosphatidylinositol (a type of phospholipid) to form 1,2-diacylglycerol and inositol phosphate, as a component of the degumming process. It will be used at the minimum level required to achieve the desired effect, in accordance with the principles of Good Manufacturing Practice (GMP).[1]

1.1 Objectives of the assessment

The objectives of this risk and technical assessment were to:

- determine whether the proposed purpose is solely a technological 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 by considering the:
  - safety and history of use of the production microorganism
  - safety of the enzyme preparation.

2 Food technology assessment

2.1 Identity of the enzyme

The applicant provided information regarding the identity of the enzyme and this has been verified using the IUBMB[2] enzyme nomenclature reference database (McDonald et al. 2009). Details of the identity of the enzyme are provided below.

Accepted IUBMB name:	phosphoinositide phospholipase C
Systematic name:	1-phosphatidyl-1D-myo-inositol-4,5-bisphosphate inositoltrisphosphohydrolase
Other names/common names:	triphosphoinositide phosphodiesterase; phosphoinositidase C; 1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase; monophosphatidylinositol phosphodiesterase; phosphatidylinositol phospholipase C; PI-PLC; 1-

[1] GMP is defined in section 1.1.2—2 of the Code as follows: ***GMP*** *or* ***Good Manufacturing Practice****, with respect to the addition of substances used as food additives and substances used as processing aids to food, means the practice of:*
(a) limiting the amount of substance that is added to food to the lowest possible level necessary to accomplish its desired effect; and
(b) to the extent reasonably possible, reducing the amount of the substance or its derivatives that:
*(i) remains as a *component of the food as a result of its use in the manufacture, processing or packaging; and*
(ii) is not intended to accomplish any physical or other technical effect in the food itself;
(c) preparing and handling the substance in the same way as a food ingredient.

[2] International Union of Biochemistry and Molecular Biology.

	phosphatidyl-D-myo-inositol-4,5-bisphosphate inositoltrisphosphohydrolase
IUBMB enzyme nomenclature:	EC 3.1.4.11
CAS number:	63551-76-8
Reaction:	1-phosphatidyl-1D-myo-inositol 4,5-bisphosphate + H2O = 1D-myo-inositol 1,4,5-trisphosphate + diacylglycerol

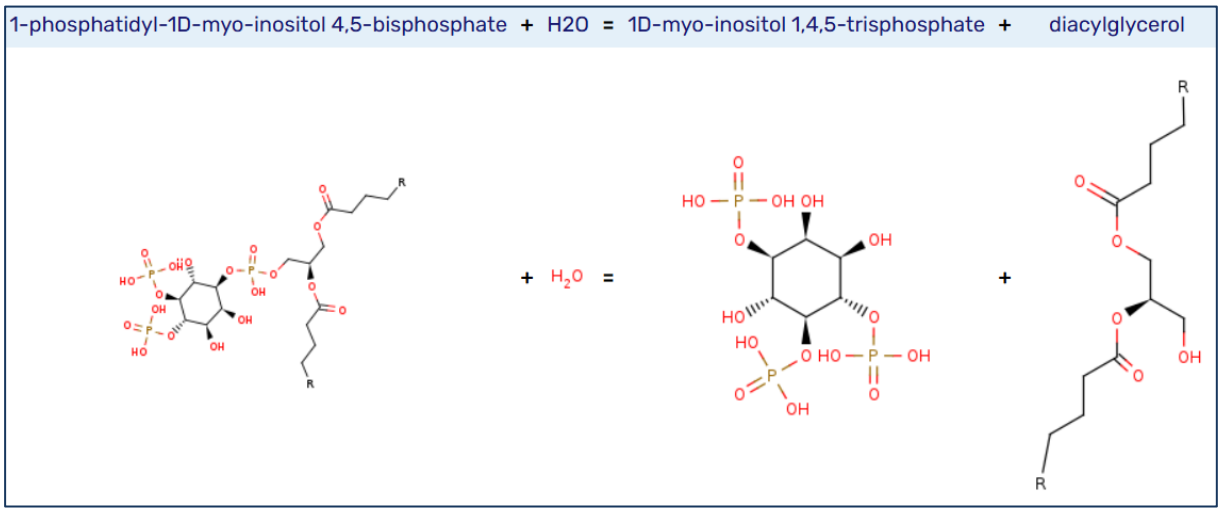

Figure 1: Reaction catalysed by phosphoinositide phospholipase C (Source: BRENDA)

2.2 Manufacturing process

2.2.1 Production of the enzyme

Enzymes from microorganisms are typically produced by controlled fermentation followed by removal of the production microorganism, purification and concentration of the enzyme. Final standardisation with stabilisers, preservatives, carriers, diluents, and other approved food-grade additives and ingredients is carried out after the purification and concentration steps.

The formulated enzymes are referred to as enzyme preparations, which, depending upon the application in food, may be a liquid, semi-liquid or dried product. Enzyme preparations may contain either one major active enzyme that catalyses a specific reaction during food processing or two or more active enzymes that catalyse different reactions (FAO/WHO 2020a).

Phosphoinositide phospholipase C is produced by submerged fed-batch pure culture fermentation of *B. licheniformis*. The fermentation process comprises three operations: injection of the stock culture suspension into the inoculum flask, seed fermentation and main fermentation.

Once fermentation is complete, a recovery process involving multiple steps to separate the *B. licheniformis* biomass from the enzyme-containing culture medium is undertaken. It includes pre-treatment with flocculants and primary separation (via drum filtration or centrifugation), followed by germ filtration (to remove residual cells and as a precaution against microbial degradation) and ultrafiltration. The product is further concentrated through the process of evaporation. This also aids in increasing the product's refractive index and increasing enzyme activity while maintaining the activity/dry matter ratio. Polyols, potassium sorbate and sodium benzoate are added to the enzyme concentrate for the purposes of

preservation and stabilisation. The typical composition of the enzyme preparation is shown in Table 1.

Table 1: Typical composition of phosphoinositide phospholipase C enzyme preparation

Component	Approximate %
Enzyme solids (Total organic solids)	0.6
Glycerol	50
Sodium benzoate	0.15
Potassium sorbate	0.1
Water	49.35

The applicant has stated the enzyme is manufactured in accordance with GMP. The quality management system used in the manufacturing process complies with ISO 9001:2015. The resultant product meets the general specifications for enzyme preparations used in food processing as established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Food Chemicals Codex (FCC).

Information on the raw materials used in producing the enzyme preparation is deemed confidential commercial information (CCI) under section 114 of the *Food Standards Australia New Zealand Act 1991* (the FSANZ Act) and can only be disclosed under certain circumstances in accordance with that Act. This information has been evaluated by FSANZ but cannot be disclosed in this report.

2.2.2 Specifications for identity and purity

There are international general specifications for enzyme preparations used in the production of food, established by JECFA in its Compendium of Food Additive Specifications (FAO/WHO 2005) and in the FCC (13th edition) (FCC 2022), referenced in section S3—2 of Schedule 3 of the Code. Enzymes used as processing aids need to meet either of these specifications, or a relevant specification in section S3—3 of Schedule 3.

Schedule 3 of the Code 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 analytical data for three representative, unstandardised batches of the enzyme preparation (section A.5 and Appendix 3.1 of the application). Table 2 provides a comparison of the summary results of those analyses with international specifications established by JECFA and the FCC, as well as those in the Code. Based on those results, the enzyme meets all relevant specifications.

In addition, the specification for identity and purity of the enzyme preparation provided by the applicant indicates an absence of the production strain.

Table 2: Analysis of applicant's liquid enzyme preparation compared to JECFA, Food Chemicals Codex, and Code specifications for enzymes

Test parameters	Test results	Specifications		
		JECFA	Food Chemicals Codex	The 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)**	ND	Absent	Negative	-
***Escherichia coli* (in 25 g)**	ND	Absent	-	-
Antimicrobial activity	ND	Absent	-	-

cfu = colony forming units; ND = not detected

2.3 Technological purpose

The phosphoinositide phospholipase C enzyme preparation is used as a processing aid in the edible oil industry for degumming fats and oils (Casado 2012). Degumming is one of the first stages of the oil refining process, carried out to reduce the content of gum impurities, including phospholipids, proteins, carbohydrates and other undesirable components that might negatively affect the quality of the final product.

When added to crude oil, phosphoinositide phospholipase C hydrolyses the phosphodiester bond of phosphatidylinositol (a type of phospholipid) at the sn-3 position, resulting in the formation of 1,2-diacylglycerol and inositol phosphate. The 1,2-diacylglycerol remains in the oil phase, whilst the inositol phosphate is solubilised in the water phase and removed from the oil by centrifugation. This reaction aids in reducing the amount of gum phospholipids in the oil, which improves physical stability, facilitates further processing and increases oil yield.

Specifically, the applicant states the benefits of using the enzyme in degumming vegetable fats and oils include:

- improved efficiency in removal of impurities (such as gums) that may affect organoleptic properties
- higher oil yield (due to significantly reduced loss of oil to gums), minimal formation of soaps and no hydrolysis of the oil
- reduced gum fraction
- improved efficiency in downstream lipid processing due to the removal of impurities
- processing cost reductions (low water consumption and reduced need for bleaching earth).

The applicant states the enzyme preparation is used at the minimum level required to achieve the desired effect, in accordance with the principles of GMP. The highest dosage given for food is 75 PLC-E[3] per kg fats and oils. This corresponds to 200 mg of the enzyme preparation per kg fats and oils and is equivalent to 1.2 mg total organic solids (TOS) per kg of fats and oils.

[3] phosphoinositide phospholipase C activity units

Phosphoinositide phospholipase C performs its primary technological function during food processing. The applicant states the enzyme exerts no function in the final food. As such, the enzyme meets the definition of a processing aid. Information on the physical and chemical properties of the enzyme preparation is summarised in Table 3.

Table 3: Phosphoinositide phospholipase C enzyme preparation physical/chemical properties

Physical/chemical properties of commercial enzyme preparation	
Enzyme activity	375 PLC-E*/g enzyme concentrate
Appearance	Liquid
Temperature range and optimum	Active up to 60°C, with an optimum of 50°C at pH 7
Thermal stability	Inactivated after 30 mins at temperatures of 60°C and above at pH 7
pH range and optimum	pH 6.8 - 9.0, with an optimum of approximately pH 7 at 30°C

*phosphoinositide phospholipase C activity units

2.4 Allergen considerations

The applicant stated the enzyme preparation does not contain known food allergens. FSANZ has reviewed the information provided in Appendix 2.1 of the application that supports this assertion.

2.5 Food technology conclusion

The use of phosphoinositide phospholipase C in degumming vegetable fats and oils is consistent with its functions as a processing aid. Its stated benefits include increased oil yields, improved physical stability and processing cost reductions. The evidence presented to support its proposed use provides adequate assurance that the use of the enzyme, in the quantity and form proposed to be used (which must be consistent with GMP), is technologically justified.

Phosphoinositide phospholipase C performs its technological purpose during the manufacture of food products and is not performing a technological purpose in the final food. This is because it will be either denatured during processing steps that involve high temperatures and/or removed in certain processing steps. It therefore functions 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 has provided evidence that the enzyme meets these specifications.

3 Safety assessment

This safety assessment aims to evaluate potential public health and safety concerns that may arise from using this phosphoinositide phospholipase C as a processing aid.

Some information relevant to this section is deemed CCI under section 114 of the FSANZ Act. This information has been evaluated by FSANZ but cannot be disclosed in this report.

3.1 Source microorganism

The host organism of the enzyme is *Bacillus licheniformis* Si3 lineage which was derived from a natural isolate of the Ca63 strain, which has a long history of safe use by the applicant for production of food enzymes. FSANZ's assessment found the name *B*. *licheniformis* is validly published under the International Code of Nomenclature of Bacteria. *B*. *licheniformis* is a Gram-positive spore-forming bacterial species of high biotechnological interest with numerous present and potential uses, including the production of bioactive compounds that are applied in a wide range of fields, such as aquaculture, agriculture, food, biomedicine, and pharmaceutical industries (Muras et al. 2021). The European Food Safety Authority (EFSA) has granted *B*. *licheniformis* with qualified presumption of safety (QPS) status (EFSA BIOHAZ Panel et al. 2025). This microorganism also falls under Class 1 Containment under the European Federation of Biotechnology guidelines (Frommer et al. 1989).

While *B*. *licheniformis* isolates have been reported to be associated with foodborne illness from cooked meats, ice cream, cheese, raw milk, infant feed, prawns (Salkinoja-Salonen et al. 1999), the incidence of human infections and pathogenicity is rare and tends to be limited to immune-compromised individuals (Haydushka et al. 2012; Logan 2012).

B. *licheniformis* is widely used to produce food-grade enzymes and other food products (Aslam et al. 2020). FSANZ has previously assessed the safety of *B*. *licheniformis* for several food processing aids. Schedule 18 of the Code currently permits the use of the following *B*. *licheniformis* produced enzyme processing aids: serine proteinase (Application A1098), subtilisin (A1206), alpha-amylase (A1219), beta-amylase (A1220) and transglutaminase (A1275).

Molecular data provided by the applicant confirmed the identity of the production organism *B*. *licheniformis*. Analysis of characteristics of three representative batches of enzyme, along with the described production methodology, demonstrated that culture conditions are applied appropriately and consistently between batches. Methodology and results confirming the production organism is not detected in the final enzyme product were provided by the applicant.

No public health and safety concerns were identified. The production organism is non-pathogenic, non-toxigenic, and does not contain any genetic material that could give rise to resistance to antibiotics.

3.2 Characterisation of the genetic modification to the production strain

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

The gene encoding the phosphoinositide phospholipase C enzyme was introduced into the genome of the host *B. licheniformis* to construct the production strain. The inserted phosphoinositide phospholipase gene is a synthetic gene. Data provided by the applicant and analysed by FSANZ confirmed the identity of the phosphoinositide phospholipase C enzyme.

The vectors used to transform the *B*. *licheniformis* recipient strain are based on *Staphylococcus aureus* standard vectors. One vector contains the phosphoinositide phospholipase C expression cassette consisting of a hybrid *Bacillus* promotor, the coding sequence for phosphoinositide phospholipase C from *Pseudomonas* sp. 62186 and a hybrid *Bacillus* terminator. A second vector was used to remove a marker gene present in the recipient strain. No elements of the vectors are left in the production strain.

The phosphoinositide phospholipase C expression cassette was integrated at specific

integration sites present in the recipient strain. A transformant was screened for rapid growth and high phosphoinositide phospholipase C activity, leading to the final production strain.

3.2.2 Characterisation of the inserted DNA

The final production strain does not contain any elements of the vectors, or any functional antibiotic resistance genes. The absence of genes of concern, and the presence of the inserted DNA, were confirmed by genome sequencing of the final production strain.

3.2.3 Stability of the introduced DNA

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

The applicant provided results of analysis of the genetic stability of the production strain, tested at large-scale fermentation. The analysis was based on phenotypic characteristics, i.e. enzyme activity and protein synthesis. No instability of the strain was observed.

3.3 Safety of the enzyme

3.3.1 History of safe use

There does not appear to be an established history of safe use for the specific phosphoinositide phospholipase C that is the subject of this application. However, phospholipases have a long history of safe use in food production. Several phospholipase A preparations have been approved for use as processing aids by FSANZ and included in Section 18 of the Code, as part of applications A501, A561, A1004, A1221 and A1246 (FSANZ 2004, FSANZ 2006, FSANZ 2009, FSANZ 2022a, FSANZ 2022b).

3.3.2 Bioinformatic assessment of homology with known toxins

A bioinformatics search was performed (February 2024) to compare the similarity of the phosphoinositide phospholipase C amino acid sequence to known toxins. The search was conducted using the National Center for Biotechnology Information (NCBI) Identical Protein Groups[4] database. No matches of concern were identified in the assessment of homology with known toxins.

3.3.3 Toxicology data

The applicant submitted unpublished toxicological studies with their phosphoinositide phospholipase C preparation which were reviewed in the present assessment:

- Bacterial reverse mutation assay
- *In vitro* mammalian cell micronucleus test
- 13-week oral toxicity study in rats.

Genotoxicity studies

Two genotoxicity studies with the phosphoinositide phospholipase C preparation were submitted. These studies were conducted in accordance with GLP and OECD Test Guidelines. The positive controls in these studies produced the expected responses. The results of these studies, as summarised in Table 4, showed no evidence of mutagenicity, clastogenicity or aneugenicity.

[4] NCBI Identical Protein Groups

Table 4: Genotoxicity studies of phosphoinositide phospholipase C from *B. licheniformis*

Test[1]	*Test object*	*Concentration*	*Purity (% total organic solids)*	*Results*	*Reference*
Bacterial reverse mutation assay (OECD TG 471, [1997])	*Salmonella typhimurium strains* TA98, TA100, TA1535 and TA1537; and *Escherichia coli* strain WP2 uvrA pKM101	Experiment I[1]: 16, 50, 160, 500, 1600 and 5000 µg TOS/plate Experiment II[1]: 51.2, 128, 320, 800, 2000, 5000 µg TOS/plate	7.3% w/w	Negative ± S9[2,3,4,5]	Ballantyne (2016)
Micronucleus tests in vitro (OECD TG 487, [2014])	Cultured human peripheral blood lymphocytes[6]	Experiment I[7, 8]: 3+21h -S9; 250, 500, 750, 1000 µg TOS/mL. 3+21h +S9; 250, 500, 750 µg TOS/mL. 24+24h -S-9; 100, 400, 600 µg TOS/mL. Experiment II: 3+21h -S9; 200, 250, 500, 750 µg TOS/mL. 3+21h +S9; 200, 250, 500, 750 µg TOS/mL.	7.3% w/w	Negative ± S9[2,9]	Whitwell (2016)

[1] Tests conducted using a modified 'treat and plate' methodology with triplicate plates for each dose and positive controls, and quintuplicate plates for vehicle controls. Plates incubated at 37±1°C for 72 hours.
[2] Absence and presence of metabolic activation by an Aroclor 1254-induced rat liver post-mitochondrial fraction (S9).
[3] No concentration-related increases in revertant numbers observed that were ≥2-fold (in strains TA98, TA100 and WP2 uvrA pKM101) or ≥3-fold (in strains TA1535 and TA1537) the concurrent vehicle control.
[4] Two replicate plate counts were above the 99% historical reference range for the vehicle control treatments with strain TA1535 in -S9 in Experiment I and II.
[5] Contamination observed on all three replicate plates treated at 50 µg TOS/plate in strain TA100 in +S9 in Experiment I. Revertant counts were comparable to data from adjacent treatment concentrations.
[6] Cultures pooled from two healthy, non-smoking male donors aged 25–33 years old for each experiment.
[7] Experiment I conducted as two trials due to a failure of the positive control in the first trial.
[8] Highest concentrations tested in Experiment I were determined following a preliminary cytotoxicity range-finder experiment.
[9] Statistically significant increases in micronucleated/binucleated cell frequency observed in Experiment I for 3+21h -S9 at 500, 750 and 1000 µg TOS/mL and for 3+21h +S9 at 750 µg TOS/mL. No significant increases in micronucleated/binucleated cells observed in Experiment II. The authors concluded the concentrations for the micronucleus analysis were limited by the presence of post-treatment precipitate in Experiment I.

Toxicity studies

13-week oral toxicity study in rats (Rees, 2017). Regulatory status: GLP; conducted in accordance with OECD Test Guideline (TG) 408 (1998)

Phosphoinositide phospholipase C was administered to male and female Han Wistar (RccHan™: WIST) rats by oral gavage at doses of 77.8, 194.5 or 505.8 mg total organic solids (TOS)/kg bw/day, equivalent to doses of 10%, 25% or 65% of the test item, respectively. The test item was a liquid concentrate prepared with reverse osmosis water at a volume-dose of 10 mL/kg bw. A control group received the vehicle at the same volume-dose. All animals survived to the end of the treatment period. Histopathological examination showed minor irritation of the stomach associated with the treatment. A higher incidence of inflammatory cell infiltrate was observed at a minimal severity in the glandular mucosa/submucosa in high-dose (505.8 mg TOS/kg bw) males, compared to controls. The

incidence and severity of inflammatory cell infiltrate of the glandular mucosa/submucosa was equivalent in mid-dose (194.5 mg TOS/kg bw) and control males. The adverse effect was observed in one high-dose female and attributed to normal biological variation in female animals, based on the incidence of mucosal infiltrate of inflammatory cells/glandular region reported in previous studies. There was no related evidence of a systemic inflammatory response or a change in markers of tissue damage observed in male or female animals. No additional treatment-related clinical signs or adverse effects on any of the parameters evaluated were observed. The no observed adverse effect level (NOAEL) in this study was 194.5 mg TOS/kg bw/day.

3.3.4 Potential for allergenicity

Searches were performed (February 2024[5]) to compare the similarity of the phosphoinositide phospholipase C amino acid sequence to known allergens. The searches were conducted using the Comprehensive Protein Allergen Resource (COMPARE)[6] database. No matches to known allergens were identified in a search for >35% identity to known allergens in the phosphoinositide phospholipase C sequence using stretches of 80 amino acids and over the full length of the alignment.

Based on the available information, the enzyme preparation is not expected to pose a risk of food allergenicity.

3.3.5 Assessments by other regulatory agencies

The safety of phosphoinositide phospholipase C from *B. licheniformis* has been evaluated by EFSA (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) et al. 2022). EFSA identified no public health and safety concerns associated with the intended use of phosphoinositide phospholipase C from *B. licheniformis*. The enzyme preparation has been approved for use as a processing aid in food in Denmark (2018) and Mexico (2019). The enzyme is also included in the positive list of approved processing aids in food in Brazil (2019) and France (2023).

The United States (US) Food and Drug Administration (FDA) has responded that it has 'no questions' to Novozymes' GRAS notification of the phosphoinositide phospholipase C enzyme preparation produced by *B. licheniformis* (FDA 2017).

4 Dietary exposure assessment

The objective of the dietary exposure assessment was to review the budget method calculation submitted by the applicant as a 'worst-case scenario' approach to estimating likely levels of dietary exposure, assuming that all of the TOS from the phosphoinositide phospholipase C enzyme preparation remained in the food.

The budget method is a valid screening tool for estimating the theoretical maximum daily intake (TMDI) of a food additive (Douglass et al. 1997). The calculation is based on physiological food and liquid requirements, the food additive concentration in foods and beverages, and the proportion of foods and beverages that may contain the food additive. The TMDI can then be compared to an acceptable daily intake (ADI) or a NOAEL to estimate a Margin of Exposure (MOE) for risk characterisation purposes. Whilst the budget method was originally developed for use in assessing food additives, it is also appropriate to use for estimating the TMDI for processing aids (FAO/WHO 2020b). The method is used by overseas regulatory bodies and JECFA (FAO/WHO 2021) for dietary exposure assessments

[5] The applicant performed the search based on data downloaded from the database in January 2024.
[6] COMPARE

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 (bw)/day
- 50% of solid food is processed
- all processed food contains 25% phosphatidylinositol (a phospholipid) (or phosphatidylinositol (a phospholipid)-derived) dry matter
- the maximum physiological requirement for liquid is 100 mL/kg bw/day (the standard level used in a budget method calculation for non-milk beverages)
- 25% of non-milk beverages are processed
- all processed beverages contain 12% phosphatidylinositol (a phospholipid) (or phosphatidylinositol (a phospholipid)-derived) dry matter
- the densities of the beverages are ~ 1
- all solid foods and non-milk beverages contain the highest use level of 1.2 mg TOS/kg in the raw material (oils and fats)
- all of the TOS from the enzyme preparation remains in the final food.

Based on these assumptions, the applicant calculated the TMDI of the TOS from the enzyme preparation to be 0.00734 TOS/kg bw/day.

As assumptions made by the applicant differ from those that FSANZ would have made in applying the budget method, FSANZ independently calculated the TMDI 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 bw/day (the standard level used in a budget method calculation where there is potential for the enzyme preparation to be in baby foods or general purpose foods that would be consumed by infants).
- FSANZ would generally assume 12.5% of solid foods contain the enzyme based on commonly used default proportions noted in the FAO/WHO Environmental Health Criteria (EHC) 240 Chapter 6 on dietary exposure assessment (FAO/WHO 2009). However, the applicant has assumed a higher proportion of 50% based on the nature and extent of use of the enzyme and therefore FSANZ has also used this proportion for solid foods as a worst-case scenario.

All other inputs and assumptions used by FSANZ remained as per those used by the applicant. The TMDI of the TOS from the enzyme preparation based on FSANZ's calculations for solid food and non-milk beverages is 0.01 mg TOS/kg bw/day.

Both the FSANZ and applicant's estimates of the TMDI will be overestimates of the dietary exposure given the conservatisms in the budget method. This includes that it was assumed that all of the TOS from the enzyme preparation remains in the final foods and beverages whereas the applicant has stated that the enzyme is either not present in the final food, or present in insignificant quantities having no function or technical effect in the final food. In

addition, the enzyme would be inactivated and perform no function in the final food to which the ingredient is added.

5 Discussion

The use of phosphoinositide phospholipase C from *B. licheniformis* containing the phosphoinositide phospholipase C gene from *Pseudomonas* sp. 62186 as a processing aid in degumming vegetable fats and oils is consistent with its known functions. It will be used in refining fats and oils where it may confer benefits including increased yields, improved physical stability and processing cost reductions.

Phosphoinositide phospholipase C is functioning as a processing aid for the purposes of the Code and does not perform a technological purpose in the food for sale. The evidence presented to support its proposed use provides adequate assurance that the use of the enzyme, in the quantity and form proposed to be used (which must be consistent with GMP), is technologically justified.

Sufficient information has been provided to assess the safety of the phosphoinositide phospholipase C that is the subject of this application.

No public health or safety concerns were identified concerning the use of the production organism, which is neither pathogenic nor toxigenic. Analysis of the production strain confirmed the presence and stability of the inserted DNA.

Phosphoinositide phospholipase C from *B. licheniformis* has been approved for use as a processing aid in the USA, Denmark, Mexico, Brazil and France.

No significant homology between the enzyme and any known toxins or allergens was identified. The enzyme preparation is not expected to pose a food allergenicity concern under the proposed conditions of use.

Phosphoinositide phospholipase C showed no evidence of genotoxicity *in vitro*. The enzyme preparation was associated with a minor irritation of the stomach in a 13-week oral toxicity study in rats. Histopathological examination indicated a higher incidence of inflammatory cell infiltrate at a minimal severity in the glandular mucosa/submucosa in high-dose (505.8 mg TOS/kg bw) males, compared to controls. No additional treatment-related clinical signs or adverse effects were observed. The NOAEL in this study was 194.5 mg TOS/kg bw/day.

The TMDI was calculated by FSANZ to be 0.01 mg TOS/kg bw/day. A comparison of the NOAEL and the TMDI results in a MOE of approximately 19,000.

Based on the large MOE, it is concluded that an ADI ‘not specified’ is appropriate.

Overall, FSANZ concludes there are no safety concerns from the use of phosphoinositide phospholipase C from *B. licheniformis* in the quantity and form required to perform its typical function in degumming vegetable fats and oils, which must be consistent with GMP.

6 References

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