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Supporting document 1

Risk and technical assessment – Application A1338

Triacylglycerol lipase from *Komagataella phaffii* (gene donor: *Yarrowia lipolytica*) for use as a processing aid

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Executive summary

Chr. Hansen Pty Ltd has applied to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of the enzyme triacylglycerol lipase (EC 3.1.1.3), from *Komagataella phaffii* containing the triacylglycerol lipase gene from *Yarrowia lipolytica.* The enzyme is proposed to be used as a processing aid to hydrolyse lipids during the manufacture of dairy-based products and plant-based dairy analogues.

The available evidence provides adequate assurance the proposed use of triacylglycerol lipase from *K. phaffii* as a processing aid is technologically justified. Triacylglycerol lipase 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.

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. Bioinformatics analysis found no significant homology between the triacylglycerol lipase enzyme and known toxins or allergens.

Studies with another enzyme preparation, BD-16449 lipase, produced by a related production strain, found no evidence of genotoxicity *in vitro* or *in vivo* and no adverse effects were observed in a 90-day oral toxicity study in rats. The no observed adverse effect level (NOAEL) was 1680 mg/kg bw/day total organic solids (TOS), the highest dose tested.

The theoretical maximum daily intake (TMDI) of this triacylglycerol lipase was calculated to be 0.45 mg TOS/kg bw/day. A comparison of the NOAEL and the TMDI results in a large Margin of Exposure (MOE) of approximately 3,700.

Based on the reviewed data it is concluded that, in the absence of any identifiable hazard, an Acceptable Daily Intake (ADI) ‘not specified’ is appropriate.

FSANZ concludes there are no public health and safety concerns associated with the use of triacylglycerol lipase from *K. phaffii* in the quantity and form required to perform its typical function in dairy and plant-based dairy analogues, which must be consistent with GMP.

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1 Introduction

Chr. Hansen 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 triacylglycerol lipase (EC 3.1.1.3) from *Komagataella phaffii* containing the triacylglycerol lipase gene from *Yarrowia lipolytica* as a processing aid in dairy and plant-based dairy analogues.

Triacylglycerol lipase will be used to hydrolyse lipids (triglycerides, diglycerides and monoglycerides) to yield free fatty acids and monoglycerides, diglycerides and glycerol. It will be used in cheese production, the production of flavouring preparations from dairy products (enzyme-modified dairy ingredients), and the production of plant-based dairy analogues. 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:	triacylglycerol lipase
Systematic name:	triacylglycerol acylhydrolase
Other names/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; GA 56;

[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.

	capalase L; triglyceride hydrolase; triolein hydrolase; tween-hydrolyzing esterase; amano CE; cacordase; triglyceridase; triacylglycerol ester hydrolase; amano P; amano AP; PPL; glycerol-ester hydrolase; GEH; meito Sangyo OF lipase; hepatic lipase; lipazin; post-heparin plasma protamine-resistant lipase; salt-resistant post-heparin lipase; heparin releasable hepatic lipase; amano CES; amano B; tributyrase; triglyceride lipase; liver lipase; hepatic monoacylglycerol acyltransferase
Commercial name:	SpiceIT® M100
IUBMB enzyme nomenclature:	EC 3.1.1.3
CAS number:	9001-62-1
Reaction:	triacylglycerol + H2O = diacylglycerol + a carboxylate

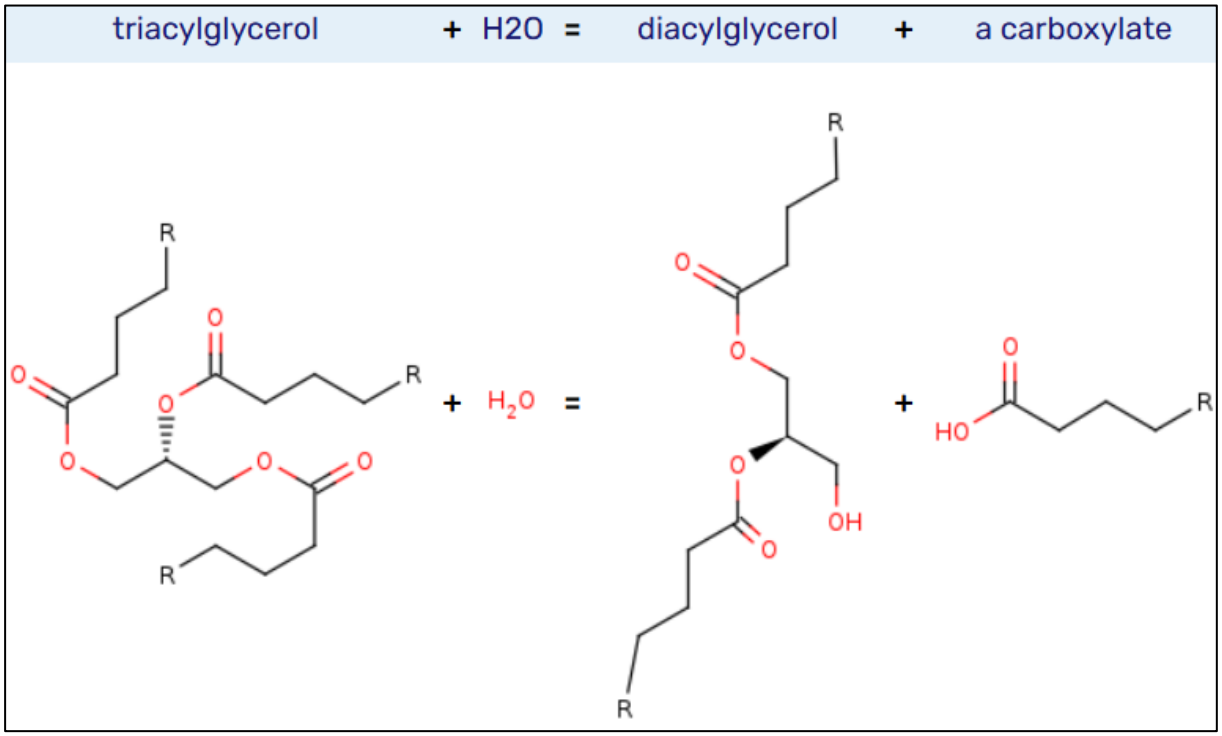

Figure 1: Reaction catalysed by triacylglycerol lipase (Source: BRENDA)

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).

Triacylglycerol lipase is produced by submerged fed-batch pure culture fermentation of *K. phaffii*. The fermentation process comprises three operations: direct inoculation of the stock culture suspension into the seed fermenter, seed fermentation and main fermentation.

Once fermentation is complete, a recovery process involving multiple steps to separate the *K. phaffii* biomass from the enzyme-containing culture medium is undertaken. It includes two phases of cross-flow microfiltration followed by polish filtration (to remove any remaining small insoluble particles or unwanted molecules). The product is adjusted with water and glycerol to reach the desired enzyme concentration.

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

Table 1: Typical composition of triacylglycerol lipase preparation

Component	Approximate %
Enzyme solids (Total organic solids)	1
Glycerol	15
Sorbitol	57
Water	27

The applicant has stated the enzyme is manufactured in accordance with GMP and the principles of Hazard Analysis of Critical Control Points (HACCP) and has provided evidence of compliance of the production site with the Food Safety System Certification 22000 (FSSC 22000) standard (see Appendix 4.1 of the application). 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) (see also section 2.2.2 of this report).

Information on the raw materials used in the production and recovery of 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 and in the FCC (13th edition), 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 standardised batches of the enzyme (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 met

the relevant specifications.

Table 2: Analysis of representative standardised batches of enzyme 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)	ND (LOQ <0.05)	≤5	≤5	≤2
Arsenic (mg/kg)	<0.1	-	-	≤1
Cadmium (mg/kg)	<0.5	-	-	≤1
Mercury (mg/kg)	<0.05	-	-	≤1
Coliforms (cfu/g)	<1	≤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; LOQ = limit of quantitation

2.3 Technological purpose

The triacylglycerol lipase enzyme preparation will be used as a processing aid to hydrolyse lipids (triglycerides, diglycerides and monoglycerides) to yield free fatty acids and monoglycerides, diglycerides and glycerol.

It will be used in cheese production, the production of flavouring preparations from dairy products (enzyme-modified dairy ingredients), and the production of plant-based dairy analogues.

Benefits of its action, as stated in the application, include:

- improved flavour of various cheese types, including piquant and butyric flavours
- development of characteristic flavours in the production of flavouring preparations from dairy products
- development of dairy-like flavours in plant-based dairy analogue products
- formation of desirable textures in plant-based dairy analogues while limiting the addition of saturated or hydrogenated fat.

Literature on food industry applications of triacylglycerol lipase support the stated function, purpose, and benefits associated with dairy products (Reyes-Reyes et al. 2022, Guerrand 2017, Chandra et al. 2020, Jooyandeh et al. 2009) and plant-based dairy analogues (Bachmann 2001, Huang et al. 2025).

The applicant stated 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 of the enzyme given for cheese production is 100 LVU per litre of milk. This corresponds to 120 mg of enzyme preparation per litre of milk and is equivalent to 1.2 mg total organic solids (TOS) per litre of milk.

Triacylglycerol lipase performs its primary technological function during food processing. The applicant states the enzyme exerts no function in the final food. This is due to a number of factors depending on the process conditions used by the individual manufacturer. These

include denaturation of the enzyme during heat treatment (e.g. pasteurisation), pH changes, depletion of substrate and physical removal. 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: Triacylglycerol lipase enzyme preparation physical/chemical properties

Physical/chemical properties of commercial enzyme preparation	
Enzyme activity	1070 LVU/g*
Appearance	Liquid
Temperature optimum	35°C, with activity decreasing above this temperature
Thermostability	Enzyme activity starts to decrease when exposed to temperatures above 35°C and becomes highly unstable when exposed to temperatures above 50°C
pH range and optimum	Range pH 3-8, peaking at pH 4-5, with an optimum of pH 4.5

*LVU = enzyme activity units, where it is understood that one LVU is the amount of enzyme that releases 1 micromole of fatty acid per minute under specified conditions (temperature, pH, substrate type). The enzyme activity of 1070 LVU/g is an average activity level across three representative batches (Table 1 of application).

2.4 Allergen considerations

The applicant provided product information which indicated that the enzyme preparation does not contain known food allergens (Appendix 2.1 of the application).

2.5 Food technology conclusion

The use of triacylglycerol lipase to hydrolyse lipids during the manufacture of dairy-based products and plant-based dairy analogues is consistent with its functions as a processing aid.

The stated benefits include the development of characteristic dairy-like flavours and a desirable texture in plant-based analogues. 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.

Triacylglycerol lipase performs its technological purpose during the manufacture of food products and is not performing a technological purpose in the final food. This is due to various factors including denaturing during processing steps that involve high temperatures and/or removal 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 enzyme 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

Komagataella phaffii, formerly known as *Pichia pastoris*, has undergone several taxonomic revisions. Although *P. pastoris* was historically treated as a single species, based on isolates first obtained in Europe and later from North America, genomic analyses demonstrated that these strains represent two distinct species (Zahrl et al. 2017). Kurtzman (2005) subsequently reassigned them as *K. phaffii* and *Komagataella pastoris*. Because of this formal taxonomic split and the resulting clarification of species boundaries, the use of the updated identifier *Komagataella phaffii* is considered the most accurate and scientifically appropriate nomenclature.

K. phaffii is a non-pathogenic, methylotrophic yeast originally isolated from black oak trees in California, USA (Bernauer et al. 2021, Heistinger et al. 2020). It has been used since the 1980s in the manufacture of pharmaceutical compounds, animal feeds, food enzymes and proteins (Anaya et al. 2024, Barone et al. 2023). This history of use includes several enzyme processing aids approved by EFSA, the US FDA and FSANZ (Spohner et al. 2015).

K. phaffii has been granted qualified presumption of safety (QPS) status by the European Food Safety Authority (EFSA) for production purposes, provided viable cells are absent from the final product (EFSA 2018, EFSA 2024). This qualification is consistent with the species' primary use as a production host rather than a food microorganism (EFSA 2018).

The host organism used by the applicant is a derivative of *K. phaffii* NRRL Y-11430. NRRL Y-11430 is a non-toxigenic and non-pathogenic strain with a demonstrated history of safe use in manufacturing proteins for use in food, feed and pharmaceuticals (Lynch et al. 2023). FSANZ previously assessed the safety of a *K. phaffii* strain derived from NRRL Y-11430 in Application A1301 and found no safety concerns associated with its use as an enzyme production organism (FSANZ 2024).

The production strain in this application was created by inserting a triacylglycerol lipase gene from *Yarrowia lipolytica* into *K. phaffii* NRRL Y-11430 (See section 3.2 Characterisation of the genetic modification to the production strain for more information). CCI information provided by the applicant confirms the production organism's identity as *K. phaffii.* The applicant also demonstrated the absence of viable cells from the production organism in the final product.

FSANZ has identified no microbiological safety concerns associated with the use of *K. phaffii* as a production organism for triacylglycerol lipase.

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 triacylglycerol lipase enzyme was synthesised based on the sequence from *Y. lipolytica* available in public databases. Data provided by the applicant and analysed by FSANZ confirmed the identity of the triacylglycerol lipase enzyme.

The gene was integrated into the *K. phaffii* genome using standard molecular biology techniques and placed under the control of native *K. phaffii* regulatory elements. Native *K. phaffii* genes were used as selectable markers to identify positive transformants.

3.2.2 Characterisation of the inserted DNA

Next generation sequencing data provided by the applicant confirmed the presence of the inserted DNA in the genome of the production strain. No antibiotic-resistance markers are present in the final production strain.

3.2.3 Stability of the introduced DNA

The assessment confirmed the inserted gene is integrated into genome of the production strain and does not have the ability to replicate autonomously. Therefore, the inserted gene is considered genetically stable.

To further demonstrate stability, the applicant provided phenotypic data from large-scale fermentation of the production strain. These data confirmed consistent expression of the triacylglycerol lipase gene across multiple generations, supporting its genetic stability.

3.3 Safety of the enzyme

3.3.1 History of safe use

The applicant stated that the triacylglycerol lipase enzyme is used during the processing of lipid-containing food in a range of countries where there are no restrictions on the use of enzyme processing aids or where the enzyme is covered by a country positive list or specific approval. The enzyme is also approved for use in Denmark. The applicant provided sales volume data as CCI demonstrating it has been sold in the USA for several years.

Triacylglycerol lipase enzymes have a long history of safe use in food production. A number of triacylglycerol lipase preparations derived from other sources have been approved for use as processing aids by FSANZ and included in Section 18 of the Code. Triacylglycerol lipase enzymes from other sources are also approved in other countries such as Brazil, Canada, China, Denmark, France, Japan and Mexico.

3.3.2 Bioinformatic assessment of homology with known toxins

The applicant conducted a search for sequence homology of triacylglycerol lipase from *K. phaffii* to known toxins in the NCBI Identical Protein Groups resource[3] in January 2025. No hits with an E-value < 0.00001 were identified, indicating the enzyme is not significantly homologous to known toxins.

3.3.3 Toxicology data

No toxicological studies with the applicant's triacylglycerol lipase enzyme are available. Studies conducted with proteins produced by related strains of *K. phaffii* were provided to support the safety of the production strain used for triacylglycerol lipase. All production strains were derived from the *K. phaffii* parental strain CBS 7435, also referred to as NRRL Y-11430. This safe strain lineage concept is consistent with Food and Agriculture Organization/World Health Organization guidance on risk assessment of food enzymes (FAO/WHO 2020c).

The studies referred to by the applicant included those assessing soy leghemoglobin, which is produced by a strain (MXY0541) derived from *K. phaffii* NRRL Y-11340. FSANZ previously evaluated the soy leghemoglobin preparation (LegH Prep) as part of Application A1186[4].

[3] https://www.ncbi.nlm.nih.gov/ipg/
[4] https://www.foodstandards.gov.au/food-standards-code/applications/A1186

LegH Prep was not genotoxic *in vitro* and did not cause adverse effects in short-term toxicity studies in rats. The no observed adverse effect level (NOAEL) of freeze-dried LegH Prep in a 28-day dietary toxicity study in rats was 1536 mg/kg bw/day, the highest dose tested. This dose corresponds to 1421 mg/kg bw/day total organic solids (TOS).

The applicant also referred to toxicity studies with a lipase enzyme and with oubli fruit sweet protein, also known as brazzein, both produced by a *K. phaffii* strain derived from the NRRL Y-11340 parent strain (Ciofalo et al. 2006, Lynch et al. 2023). Both test items showed no evidence of genotoxicity, and no adverse effects were observed in 90-day oral toxicity studies in rats.

Based on the available information, the test item used in the toxicity studies of lipase produced by *K. phaffii* strain DVSA-PLC-004, derived from the wild-type strain NRRL Y-11340, is considered suitably equivalent for assessing the safety of the *K. phaffii* production strain and the lipase enzyme that is the subject of this application. The studies with the lipase enzyme are summarised below.

Toxicology

90-day oral toxicity study in rats (Ciofalo et al. 2006; Ciofalo et al, 2008) Regulatory status: not reported

DV16449 lipase produced by *K. phaffii* DVSA-PLC-004 was administered to Sprague-Dawley rats (20/sex/group) by oral gavage at doses of 0, 420, 840 or 1680 mg/kg bw/day TOS for 90 days. Mortality and clinical observations were evaluated daily. Body weight and food consumption were recorded weekly. Ophthalmology examinations were performed before treatment and during the last week of treatment. At the end of the study blood samples were collected for haematology, coagulation and clinical chemistry analyses. Selected tissues from the control and high dose groups were weighed at necropsy and evaluated microscopically.

Three animals were found dead during the study (dose groups not reported), but all were attributed to gavage incidents. No clinical signs of toxicity were observed. There were no treatment-related adverse effects on body weight, food consumption, ophthalmology, haematology, coagulation, clinical chemistry, organ weights and histopathology. The NOAEL was 1680 mg/kg bw/day TOS, the highest dose tested.

Genotoxicity

Genotoxicity studies with BD16449 lipase produced by *K. phaffii* strain DVSA-PLC-004, derived from *K. phaffii* NRRL Y-11340, comprised a bacterial reverse mutation test, an *in vitro* chromosomal aberration assay and micronucleus assay in mice.

The GLP status of the test laboratory, and the test guidelines followed, were not reported. However, the protocols were consistent with those of the relevant OECD test guidelines.

The results of these studies, as summarised in Table 4, demonstrate that lipase produced by *K. phaffii* strain DVCSA-PLC-004 does not pose a concern for mutagenicity, clastogenicity and aneugenicity.

Table 4: Genotoxicity studies with BD16449 lipase

Test	Test object	Concentration	Results	Reference
In vitro				
Bacterial reverse	*Salmonella typhimurium*	Salmonella strains: 0, 154,	Negative ± S9	Ciofalo et al. 2006

Test	Test object	Concentration	Results	Reference
mutation test (treat and plate method)	strains TA98, TA100, TA1535 & TA1537; *Escherichia coli* strain WP2*uvrA* (pKM101)	512, 1540, 5120, or 7690 µg/mL; equivalent to 0, 100, 333, 1000, 3330 or 5000 µg/plate WP2*uvr*A: 0, 15.4, 51.2, 154, 512, 1540, 5120 or 7690 µg/mL; equivalent to 0, 10, 33.3, 100, 333, 1000, 3330 or 5000 µg/plate		
In vitro mammalian cell chromosomal aberration assay	Human peripheral blood lymphocytes	Initial assay (3h treatment): - S9: 0, 412, 1200, 2450 or 5000 µg/mL; + S9 0, 588, 1200, 2450 or 5000 µg/mL Confirmatory assay (22h treatment – S9, 3h treatment + S9): - S9: 0, 2000, 3000, 4000 or 5000 µg/mL	Negative ± S9	Ciofalo et al. 2006
In vivo				
Mouse micronucleus assay	CD-1(ICR)BR mice	24 h analysis: 0, 500, 1000 or 2000 mg/kg bw; 48h analysis: 0 or 2000 mg/kg bw	Negative	Ciofalo et al. 2006

3.3.4 Potential for allergenicity

The potential allergenicity of the applicant's triacylglycerol lipase was assessed by comparing its amino acid sequence with those of known allergens. The applicant conducted the following searches in October 2024 using the COMprehensive Protein Allergen REsource (COMPARE[5]):

- 35% identity over 80 amino acids
- 35% identity over 80 amino acids with scaling enabled
- Full length alignment
- 100% identity over 8 contiguous amino acids

[5] https://comparedatabase.org/

FSANZ also conducted searches using the Allergen Online database[6].

No matches with known allergens were found. A literature search found no reports of food allergy to triacylglycerol lipase enzymes.

Based on the available information, triacylglycerol lipase from *K. phaffii* is unlikely to pose a food allergenicity concern.

3.3.5 Assessments by other regulatory agencies

The Danish Veterinary and Food Administration has approved the use of triacylglycerol lipase from *K. phaffii* for use in production of cheese, plant-based analogues of milk and milk products and flavouring preparations. Information provided by the applicant indicates that this approval was based on a safety assessment made in accordance with guidelines from the European Food Safety Authority (EFSA) on submission of a dossier on food enzymes.

In the USA, the Food and Drug Administration (FDA) has responded that it has 'no questions' to Chr Hansen's Generally Recognized as Safe (GRAS) conclusion for lipase from *Y. lipolytica* expressed in *K. phaffii* (GRN No. 1201[7]). FSANZ notes that 'no questions' responses are not in themselves a safety assessment by the US FDA.

4 Dietary exposure assessment

The objective of the dietary exposure assessment was to review the budget method calculation presented by the applicant as a 'worst-case scenario' approach to estimating likely levels of dietary exposure, assuming that all of the TOS from the triacylglycerol lipase 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 for processing aids.

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

- the maximum physiological requirement for solid food (including milk) is 50 g/kg body weight/day
- 25% of all solid foods contain this triacylglycerol lipase
- the highest dosage given for cheese production is 1.2 mg TOS/kg raw material (milk)
- 10 litres of milk produces 1 kg of cheese
- all solid foods contain the highest use level of 12 mg TOS/kg in the final food (cheese)
- 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)

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

[7] https://hfpappexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=1201&sort=GRN_No&order=DESC&startrow=1&type=basic&search=lipase

- 25% of all non-milk beverages contain this triacylglycerol lipase
- the densities of non-milk beverages are ~1 g/mL
- all non-milk beverages contain the highest use level of 12 mg TOS/kg in the final food
- 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 0.45 mg TOS/kg bw/day.

Assumptions made by the applicant do not differ from those that FSANZ would have made in applying the budget method, which are conservative and reflective of a first tier in estimating dietary exposure. Hence, FSANZ did not recalculate the TMDI using different inputs or assumptions.

The 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. The applicant states that there will be no enzymatic activity in the final foods due to processing steps as chosen by the food producer, such as denaturing during heat processing, pH changes, digestion by proteases, and physical removal.

5 Discussion

The use of triacylglycerol lipase from *K. phaffii* containing the triacylglycerol lipase gene from *Y. lipolytica* as a processing aid in dairy and plant-based dairy analogues is consistent with its known functions. It will be used to hydrolyse lipids (triglycerides, diglycerides and monoglycerides) to yield free fatty acids and monoglycerides, diglycerides and glycerol. The enzyme is intended to be used in cheese production, the production of flavouring preparations from dairy products (enzyme-modified dairy ingredients), and the production of plant-based dairy analogues, where it may confer benefits including the development of characteristic dairy-like flavours and a desirable texture in plant-based analogues.

Triacylglycerol lipase 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.

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.

There is a history of use of triacylglycerol lipase from *K. phaffii* overseas, including in Denmark and the USA. No significant homology between the enzyme and any known toxins or allergens was identified. Studies with another enzyme, BD-16449 lipase, produced by a related production strain found no evidence of genotoxicity *in vitro* or *in vivo* and no adverse effects were observed in a 90-day oral toxicity study in rats. The NOAEL was 1680 mg/kg bw/day TOS, the highest dose tested. These findings support the safety of the triacylglycerol lipase production strain.

The TMDI of this triacylglycerol lipase was calculated to be 0.45 mg TOS/kg bw/day. A comparison of the NOAEL and the TMDI results in a large MOE of approximately 3,700.

Based on the reviewed data it is concluded that, in the absence of any identifiable hazard, an ADI 'not specified' is appropriate.

FSANZ concludes there are no safety concerns from the use of triacylglycerol lipase from *K. phaffii* in the quantity and form required to perform its typical function in dairy and plant-based dairy analogues, which must be consistent with GMP.

6 References

Anaya Y, Rosario Martinez R, Goodman RE, Johnson P, Vajpeyi S, Lu X, Peterson R, Weyers SM, Breen B, Newsham K, Scottoline B, Clark AJ, Malinczak C-A (2024) Evaluation of the potential food allergy risks of human lactoferrin expressed in Komagataella phaffii. Frontiers in Immunology, Volume 15 - 2024.doi:10.3389/fimmu.2024.1380028

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