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

27 July 2021
164-21

Supporting document 1

Technical and safety assessment – Application A1211

Maltogenic alpha amylase enzyme from GM *Bacillus licheniformis*

Executive summary

Danisco New Zealand Ltd applied to Food Standards Australia New Zealand (FSANZ) to amend Schedule 18 – Processing Aids of the Australia New Zealand Food Standards Code (the Code) to include maltogenic alpha-amylase (EC 3.2.1.133) from a genetically modified (GM) strain of *Bacillus licheniformis.* The source organism for the enzyme gene is *Geobacillus stearothermophilus*. The enzyme is proposed to be used as a processing aid in baking, brewing, potable alcohol production and starch processing.

The evidence presented to support the proposed use of the enzyme provides adequate assurance that the enzyme, in the quantity and form proposed to be used, is technologically justified and has been demonstrated to be effective in achieving its stated purpose. The enzyme meets international purity specifications and has been authorised for use in the USA, Denmark, Brazil and Singapore.

The assessment concluded that the use of the enzyme under the proposed conditions is safe. The host is neither pathogenic nor toxigenic and has a long history of safe use in food. The gene donor organism has a history of safe use for food enzymes and raises no safety concerns. The maltogenic alpha-amylase from *G. stearothermophilus* is already permitted in the Code, produced by another GM host. The GM production strain was confirmed to contain the inserted DNA and this DNA was shown to be inherited across several generations.

The no observed adverse effect level (NOAEL) in a 13-week repeated dose oral toxicity study in rats was the highest dose tested and corresponds to 80 mg /kg bw/day total organic solids (TOS). The theoretical maximum daily intake (TMDI) was calculated by FSANZ to be 0.31 mg/kg bw/day TOS. Comparison of the NOAEL and the TMDI gives a Margin of Exposure of more than 250.

Bioinformatic analysis showed that the enzyme has a degree of homology with several known allergens. None were food allergens. No reports of sensitisation to any form of maltogenic alpha-amylases was found in a search of the scientific literature and maltogenic alpha-amylase from the same source organism is already permitted in the Code. On that basis, this enzyme is unlikely to pose an allergen risk to consumers when used as a processing aid in food.

The enzyme formulation may contain wheat products. Wheat components are known food allergens.

Based on the reviewed toxicological data and dietary exposure data, it was concluded that an acceptable daily intake (ADI) ‘not specified’ is appropriate.

Table of contents

1 Introduction

Danisco New Zealand Ltd has applied to FSANZ, seeking permission for use of a new source of maltogenic alpha-amylase (EC 3.2.1.133) to be used as a processing aid in baking, brewing, potable alcohol production and starch processing. This enzyme is produced by a genetically modified strain of *Bacillus licheniformis* expressing the maltogenic alpha-amylase gene from *Geobacillus stearothermophilus*.

The function of the enzyme is to catalyse the hydrolysis of starch polysaccharides. That is, it hydrolyses (1→4)-alpha-D-glucosidic linkages in polysaccharides, to remove successive alpha-maltose residues from the non-reducing ends of the chains. If permitted following a pre-market assessment, the maltogenic alpha-amylase will provide an additional option for manufacturers of these different food products.

1.1 Objectives of the assessment

The objectives of this Risk and Technical Assessment for maltogenic alpha amylase were to:

- determine whether the proposed purpose is clearly stated and that the enzyme achieves its technological function in the quantity and form proposed to be used as a food processing aid
- evaluate any potential public health and safety issues that may arise from the use of this enzyme protein, produced by a GM organism as a processing aid. Specifically by considering the:
    - history of use of the host and gene donor organisms
    - characterisation of the genetic modification(s), and
    - safety of the enzyme protein.

2 Food technology assessment

2.1 Characterisation of the enzyme

2.1.1 Identity and properties of the enzyme

The production microorganism of the enzyme is a GM strain of *B. licheniformis*. The donor microorganism of the maltogenic alpha amylase gene is *G. stearothermophilus* (further details contained in section 3).

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

Table 1: Identity and relevant details of the enzyme maltogenic alpha-amylase

Generic common name:	Maltogenic alpha-amylase
Accepted IUBMB[1] name:	Glucan 1,4-alpha-maltohydrolase
Systematic name:	4-alpha-D-glucan alpha-maltohydrolase
Other names:	1,4-alpha-D-glucan alpha-maltohydrolase

[1] International Union of Biochemistry and Molecular Biology

EC number:	3.2.1.133
CAS[2] registry number:	160611-47-2
Reaction:	Hydrolysis of (1→4)-α-D-glucosidic linkages in polysaccharides so as to remove successive α-maltose residues from the non-reducing ends of the chains
Optimal temperature (°C), (range) and at maximum activity (at pH 5.2):	(40-50), 50 Activity reduced significantly at 60
Optimal pH, (range) and at maximum activity (at 50°C):	(4.4 - 6.8), 5.5 Negligible activity at pH 3.6
Stability, enzyme preparation, 20°C	6 months (of an indicative enzyme preparation)

2.2 Manufacturing process

2.2.1 Production of the enzyme

The enzyme is produced by a submerged fermentation process, which is the common production method of producing food enzymes. The specific processes are provided in the application which is summarised briefly here as these are very well known processes. They are fermentation, separation of the bacterium after completion of fermentation, autolysis to release the enzyme, separation, purification and concentration of the enzyme using filtration processes. The enzyme preparation is then standardised and stabilised with diluents to the appropriate product specification before undergoing a final polish filtration and packaging. The final enzyme preparation is an off-white powder. It is noted that some of the diluents used are derived from wheat.

The manufacturing processes ensures the production microorganism is removed from the final enzyme preparation. The final enzyme preparation is produced to ensure it complies with international purity specifications of enzymes, being the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2006) and the Food Chemicals Codex (FCC) (USP, 2018) as discussed in the next section.

2.2.2 Specifications

There are international specifications for enzyme preparations used in food production (JECFA 2006; USP 2018). Both of these specification sources are primary sources listed in section S3—2 of the Code. Enzyme preparations must meet these specifications.

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

[2] Chemical Abstracts Service

Table 2: Product specifications for commercial enzyme preparation

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

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

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

2.3 Technological purpose of the enzyme

A currently permitted form of the enzyme is approved for use in the manufacture of all foods since it is listed in subsection S18—4(5). The technological purpose of this enzyme is similar to that of another form of the enzyme which is currently being assessed (application A1210), in that it will be used in the manufacture of bakery products (FSANZ 2021). However, this form of the enzyme is proposed to also be used as a processing aid in brewing, potable alcohol production and starch processing.

As identified by the IUBMB (2017), maltogenic alpha-amylase catalyses the hydrolysis of 1-4-alpha-glucosidic linkages in polysaccharides to remove successive alpha-maltose residues from the non-reducing ends of these chains. In general the use of the enzyme is to assist in the hydrolysis of large polysaccharides like starch into smaller molecules which is of assistance in the processing of food raw materials. The summary of the technological purpose and hence benefit of using the enzyme in the manufacture of different food types is provided below.

Baking

The action of the enzyme produces smaller molecules, being mainly maltose. The formation of molecules of smaller chain lengths interrupts the usual staling process of the formation of a stable network structure that increases crumb firmness, as an indicator of staling. Similarly, an outcome is to maintain crumb softness and the resilience of the bread for longer and also ensures a uniform volume and crumb structure of the baked product.

Brewing

- Higher brewing yields due to improved conversion of starch into fermentable sugars.
- Increased flexibility in the choice of raw materials (higher proportion of raw grain to malt ratio).
- More uniform formation of fermentable sugars so less product variation.

Starch processing

- Conversion of liquefied starch into maltose rich solution.
- More specific and efficient processing compared to using acid to catalyse the hydrolysis.

Potable alcohol production

- Similar to benefits outlined for brewing, since the initial processing is similar.
- Potential higher yields of alcohol due to improved processing and more consistent conversion of starch into fermentable sugars.

2.4 Technological justification of the enzyme

Information was provided in the application supporting the benefits of using the enzyme in baking, brewing, starch processing and potable alcohol production. The specific benefits observed and identified are summarised in Table 3 below.

Table 3: Technological justification and benefits of using the enzyme in baking, brewing, potable alcohol production and starch processing

Area of use	Benefit
Baking	Improved measurement of softness of manufactured bread over the shelf life of 14 days.
Brewing	Increased concentration of fermentable sugars (especially maltose, DP2 sugars) in the mash which improves the efficacy of brewing.
Starch processing	Increased formation of maltose from the hydrolysis of liquefied starch.
Potable alcohol production	Increased concentration of fermentable sugars (especially maltose, DP2 sugars) in the mash which improves the efficacy of the subsequent distillation process.

2.5 Food technology conclusion

FSANZ concludes that the stated purpose of this enzyme preparation as a processing aid in baking, brewing, potable alcohol production and starch processing is clearly articulated in the application. The evidence presented to support the proposed uses provides adequate assurance that the enzyme, in the form and prescribed amounts, is technologically justified and has been demonstrated to be effective in achieving its stated purpose. The enzyme performs its technological purpose during production and manufacture of foods after which it is inactivated thereby not performing a technological function in the final food. It is therefore appropriately categorised as a processing aid and not a food additive. The enzyme preparation meets international purity specifications.

3 Safety assessment

Maltogenic alpha amylase is an enzyme processing aid produced by microbial fermentation. The production strain is a genetically modified bacterium. The purpose of the assessment is to examine the production strain and the final food product to identify and evaluate any safety concerns. Some information used in this assessment, including details around the genetic modification, have been supplied as confidential commercial information. This information cannot be provided in this document.

3.1 History of use

3.1.1 Host organism

B. *licheniformis* is widely used to produce food-grade enzymes and other food products (de Boer et al. 1994, Schallmey et al. 2004). FSANZ has previously assessed the safety of *B*. *licheniformis* as the source organism for a number of food processing aids (both GM and non-GM). Schedule 18 to Standard 1.3.3 of the Code currently permits the use of the following enzymes derived from *B*. *licheniformis*: alpha-amylase, chymotrypsin, endo-1,4-beta-xylanase, β-galactosidase, glycerophospholipid cholesterol acyltransferase, maltotetraohydrolase, pullulanase and serine proteinase.

Virulence is also not generally associated with *B*. *licheniformis*. There are, however, strains of *B*. *licheniformis* that have been implicated in human infection, including septicaemias in immunocompromised individuals (de Boer et al. 1994; EPA 1997). de Boer notes that case histories involving *B. licheniformis* involve a prior tissue injury, intravenous injection or catheter implantation. Toxin-producing isolates of *B*. *licheniformis* have been isolated from raw milk, commercially-produced baby food and other foods involved in food poisoning incidents (Salkinoja-Salonen et al. 1999).

The production strain relevant to this application is derived from the *B*. *licheniformis* Bra7 lineage. The applicant has used *B*. *licheniformis* production strains derived from this lineage safely for many years. Data provided with a previous enzyme application (A1164) from the same applicant confirmed the identity of the parental strain as *B*. *licheniformis*.

Modifications were made to the parental *B. licheniformis* strain to prepare an appropriate recipient strain for introducing the novel maltogenic alpha-amylase gene. The modifications include a series of genetic modification steps. A description of these changes was provided and has been assessed. No risks were identified.

3.1.2 Gene donor organism(s)

The gene donor organism, *Geobacillus stearothermophilus* has a history of safe use for the production of food enzymes including in Schedule 18 to Standard 1.3.3 of the Code. Information provided was used to confirm the identity of the donor gene.

3.2 Characterisation of the genetic modification

3.2.1 Description of DNA to be introduced and method of transformation

A typical expression cassette was generated, containing the maltogenic alpha-amylase gene flanked by a specific promoter and terminator. The sequence of the gene encodes a mature protein identical to the protein found in the donor organism.

3.2.2 Characterisation of inserted DNA

Established molecular biology methods such as genomic sequencing, were used to characterise the insertion of the expression cassette in the production strain MDT 06-221. The evidence confirmed the enzyme gene had been integrated into the genome of the host, had the expected sequence and had not undergone rearrangement.

3.2.3 Genetic stability of the inserted gene

A genotypic analysis was performed on MDT 06-221, comparing sequences before and after a model fermentation run. The results provided indicate the expression of the gene was

consistent across the generation number seen over a typical fermentation run, indicating the production strain is genetically stable.

3.3 Safety of maltogenic alpha-amylase

3.3.1 History of safe use of the enzyme

Maltogenic alpha-amylase isolated directly from *G. stearothermophilus* has been used since the mid-1990s in baking (Derde et al., 2012; Goesaert et al., 2009). Further, *G. stearothermophilus* maltogenic alpha-amylase has been assessed for use in Australia and New Zealand when manufactured using genetically modified *B. subtilis* or genetically modified *Saccharomyces cerevisiae*[3]. The subject *G. stearothermophilus* maltogenic alpha-amylase enzyme of this application is produced in *B. licheniformis* and the mature enzyme has an identical sequence to the *G. stearothermophilus* maltogenic alpha-amylases already approved.

There are no reports of adverse effects arising from the use of *G. stearothermophilus* maltogenic alpha-amylase enzymes in Australia or any other jurisdiction where the enzyme has been approved.

3.3.2 Bioinformatics concerning potential for toxicity

A BLAST search was performed using the mature amino acid sequence alpha-amylase mature protein sequence against the complete UniProt database[4]. With a conservative E-value[5] threshold of 0.1, the top 1000 matches were to maltogenic α -amylases, alpha-amylases, cyclomaltodextrin glucanotransferases or other related enzymes and isoforms found across various species. No toxins or venoms were present in these results.

In addition, A BLAST search was performed using the mature amino acid sequence alpha-amylase mature protein sequence against the UniProt animal toxin database[6]. No matches to toxins or venoms were found.

3.3.3 Evaluation of enzyme toxicity studies

The *G. stearothermophilus* maltogenic alpha-amylase test item used in the following toxicity studies was produced using *B. licheniformis* MDT 06-221 and was representative of the material that is the subject of this application.

Animal Studies

90-day repeated dose oral toxicity study in rats (Harlan Laboratories Ltd, 2011). Regulatory Status: GLP; conducted according to OECD Test Guideline 408.

The maltogenic alpha-amylase test item was administered to Wistar Han™ RccHan™:WIST rats (10/sex /group) at doses of 0, 20, 40 and 80 mg/kg bw/day TOS by oral gavage for 13 weeks. The vehicle control was 0.9% saline. Animals were housed in groups of three or four by sex, with *ad libitum* access to food and water.

[3] Application A1210, is running concurrently with this application.
[4] UniProt database: https://www.uniprot.org/
[5] The E value (or Expect value) indicates the significance of a match found when searching a sequence database. The closer an E value gets to zero, the less likely an alignment could have been produced by chance.
[6] UniProt toxins database: https://www.uniprot.org/program/Toxins

Mortality and morbidity were checked daily. Body weight, food consumption and detailed clinical examinations for signs of toxicity were recorded weekly. Ophthalmological examination was conducted on all test animals prior to treatment and on high-dose and control animals at study termination. Functional performance and sensory reactivity tests were performed in week 12. Gross pathology and measurement of organ weights was conducted on all animals at study termination, and a histopathological examination was conducted on organs and tissues from the control and high-dose group animals.

No mortality occurred during the study. No treatment related effects were observed on feed consumption, body weights, haematology, clinical chemistry, ophthalmology, or functional observations or motor activity parameters. No treatment-related macroscopic abnormalities or histopathologic findings in any of the test animals. No remarkable macroscopic or histopathological changes were observed at necropsy.

The NOAEL was set at 80 mg/kg bw/day TOS, which was the highest dose tested.

Genotoxicity

Bacterial reverse mutation test (Harlan Laboratories Ltd, 2011). Regulatory Status: GLP; conducted according to OECD Test Guideline 471.

The potential mutagenicity of maltogenic alpha-amylase was evaluated in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* strain WP2 *uvrA*, with and without metabolic activation using rat liver homogenate (S9). Mutation tests were conducted just once in triplicate, over a dose range of 50-5000 µg protein/plate, using either direct incorporation or pre-incubation.

Positive controls in the absence metabolic activation were *N*-ethyl-*N*'-nitroguanidine (WP2uvrA, TA100 and TA1535), 9-aminoacridine (TA1537) and 4-nitroquinoline-1-oxide (TA98). Positive controls in the presence of metabolic activation were 2-aminoanthracene (WP2uvrA, TA100, TA1535 and TA1537) and benzo[a]pyrene (TA98). Sterile deionised water was used as the vehicle control.

No concentration-related increases in revertant colonies were observed in cultures treated with the test item, with or without metabolic activation. All positive control treatments showed the anticipated increases in mutagenic activity demonstrating the validity of the assay. It was concluded that maltogenic alpha-amylase test item was not mutagenic under the conditions of this test.

In vitro mammalian chromosomal aberration test (Harlan Laboratories Ltd, 2011). Regulatory status: GLP; conducted according to OECD test guideline 473.

The potential of maltogenic alpha-amylase to cause chromosomal aberrations in mammalian cells was tested using human lymphocytes isolated from peripheral blood, collected from a healthy volunteer. Treatment with the maltogenic alpha-amylase test item was either a 4 hour pulse exposure with or without S9, followed by a 20 hour recovery; or 24 hours of continuous exposure. Positive control assays were conducted in parallel using mitomycin C in the absence of S9 and cyclophosphamide in the short-term treatment with S9.

Dose selection experiments observed cytotoxic activity at a total protein concentration of 78.13 µg/mL of maltogenic alpha-amylase in the 24 hour treatment. As a result, the total protein concentration for the 24 hour continuous treatment was adjusted to: 6.25-100 µg/mL total protein, while the dose range was used for the 4 hour treatments with and without S9 was retained at 78.1 - 5000 µg/mL total protein.

There were no treatment related increases in chromosomal aberration observed in peripheral blood lymphocytes following exposure to the maltogenic alpha-amylase test item, relative to the vehicle controls, under any of the conditions tested. The positive controls demonstrated a statistically significant increase in chromosomal aberration, validating the sensitivity of the experimental methodology. It was concluded that maltogenic alpha-amylase did not cause chromosome aberrations in peripheral blood lymphocytes, under the conditions of the study.

3.3.5 Potential for allergenicity

A FASTA search was performed using the mature amino acid sequence of *G. stearothermophilus* maltogenic alpha-amylase using the AllergenOnline[7] database (queried in January 2014) and the Allermatch[8] database (queried in November, 2014). Both databases were queried using three sequence alignments: full length protein (E-value 0.1), an 80 mer sliding window (>35% homology) and an 8 mer sliding window search (100% homology).

The applicant supplied allergen searches identified three known respiratory allergens with a greater than 35% sequence identity using an 80-amino acid sliding window. These allergens were: taka-amylase A (Asp o 21) from *Aspergillus oryzae*; glycoside hydrolase (Sch c 1) from *Schizophyllum commune*; and alkaline protease (Asp f 13) from *Aspergillus fumigatus*.

FSANZ updated both searches in October 2020 and identified a number of additional allergens that fulfilled the search criteria. These allergens were: probable maltase (Aed a 4) *from Aedes aegypti*; alpha-amylase (Blo t 4) from *Blomia tropicalis*; allergen Aca s 4 from *Acarus siro*; alpha-amylase (Per a 11) from *Periplaneta americana*; allergen Der f 4 from *Dermatophagoides farina*; and alpha-amylase (Bla g 11) from *Blattella germanica.*

None of the identified allergens are recognised food allergens[9]. Respiratory sensitisation of occupationally exposed individuals to some food enzyme processing aids, such as alpha-amylase and other glycoside hydrolase enzymes has been reported (Baur & Posch, 1998). However, food enzyme processing aids that are respiratory allergens are not usually food allergens (Poulsen 2004, Bindslev-Jensen et al. 2006), and there are no reports of sensitisation to maltogenic alpha-amylases in the scientific literature.

It is concluded that the presence of maltogenic alpha-amylase produced using modified *B. licheniformis* in food is unlikely to pose an allergenicity concern to consumers.

Soy products are used in fermentation of *B. licheniformis* MDT 06-221, but were undetectable by ELISA (limit of detection is 2.5 ppm) in the final enzyme product. The fermentation medium and final enzyme product contains wheat starch and wheat flour as part of the formulation.

3.3.6 Assessments by other regulatory agencies

A letter of approval for the enzyme from the Ministry of Environment and Food in Denmark was provided by the applicant.

The applicant provided a generally recognised as safe (GRAS) expert opinion, which are not assessments by the FDA and not accepted by FSANZ as an assessment by other international agencies.

---

[7] AllergenOnline: http://www.allergenonline.org/
[8] Allermatch: http://www.allermatch.org/index.html
[9] World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee: http://www.allergen.org/

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 maltogenic alpha-amylase 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 ADI or a NOAEL to estimate a margin of exposure for risk characterisation purposes.

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

- the maximum physiological requirement for solid food (including milk) is 25 g/kg body weight/day
- the maximum physiological requirement for non-milk beverages is 100 mL/kg body weight/day (the standard level used in a budget method calculation)
- 50% of solid food and 25% of non- milk beverages contain maltogenic alpha-amylase
- the maximum maltogenic alpha-amylase level in final solid food was 3.6 mg TOS/kg food and for non-milk beverage was 8.87 mg TOS/kg food (i.e. the highest use level from all uses within each group)
- all of the enzyme will remain in the final food, except for distilled beverages due to processing, however other uses in non-milk beverages were still assumed to contain the enzyme.

Based on these assumptions, the applicant calculated the TMDI of maltogenic alpha-amylase to be 0.266 mg TOS/kg body weight/day.

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

- 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, which for this enzyme would be from the bakery and starch processing uses)
- 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 as a worst case scenario.

All other inputs and assumptions used by FSANZ remained as per those used by the applicant. The TDMI based on FSANZ's calculations for solid food and non-milk beverages were 0.009 mg TOS/kg body weight/day and 0.22 mg TOS/kg body weight/day respectively, a total of 0.31 mg TOS/kg bw/day.

Both the FSANZ and applicants estimates of the TMDI will be overestimates of the dietary exposure given the conservatisms in the budget method. This includes that it was assumed that the enzyme remains in the final foods and beverages whereas the applicant has stated that it is likely to either be removed during processing or would be present in insignificant quantities, and would be inactivated and perform no function in the final food.

4 Summary

No safety concerns were identified in the assessment of the maltogenic alpha-amylase produced by microbial fermentation, using a genetically modified strain of *B. licheniformis*, under the proposed use conditions.

The host is neither pathogenic nor toxigenic and has a long history of safe use in food. The gene donor organism has a history of safe use for food enzymes and raises no safety concerns. The maltogenic alpha-amylase from *G. stearothermophilus* is already permitted in the Code, expressed from another GM host. The GM production strain was confirmed to contain the inserted DNA and this DNA was shown to be inherited across several generations.

The no observed adverse effect level (NOAEL) in a 13-week repeated dose oral toxicity study in rats was the highest dose tested and corresponds to 80 mg /kg bw/day total organic solids (TOS). The theoretical maximum daily intake (TMDI) was calculated by FSANZ to be 0.31 mg/kg bw/day TOS. Comparison of the NOAEL and the TMDI gives a Margin of Exposure of more than 250.

Bioinformatic analysis showed that the enzyme has a degree of homology with several known allergens. None were food allergens. No reports of sensitisation to any form of maltogenic alpha-amylases was found in a search of the scientific literature and maltogenic alpha-amylase from the same source organism is already permitted in the Code. On that basis, this enzyme is unlikely to pose an allergen risk to consumers when used as a processing aid in food.

The enzyme formulation may contain wheat products. Wheat components are known food allergens.

Based on the reviewed toxicological data and dietary exposure data, it was concluded that an acceptable daily intake (ADI) ‘not specified’ is appropriate.

5 References

Baur X, Posch A (1998) Characterized allergens causing bakers' asthma. Allergy 53:562–566

Bindslev-Jensen C, Skov PS, Roggen EL, Hvass P, Brinch DS (2006) Investigation on possible allergenicity of 19 different commercial enzymes used in the food industry. Food and Chemical Toxicology 44: 1909-1915

de Boer AS, Priest F, Diderichsen B (1994) On the industrial use of *Bacillus licheniformis*: a review. Appl Microbiol Biotechnol 40:595-598

Derde LJ, Gomand SV, Courtin CM, Delcour JA (2012) Characterisation of three starch degrading enzymes: thermostable β-amylase, maltotetraogenic and maltogenic alpha-amylases. Food Chem 135: 713-721

EPA (1997) *Bacillus licheniformis* final risk assessment. United States Environmental Protection Agency. https://www.epa.gov/sites/production/files/2015-09/documents/fra005.pdf Accessed 18 June 2021

FAO/WHO (2009). Chapter 6: Dietary exposure assessment of chemicals in food. In: Principles and methods for the risk assessment of chemicals in food. Rome: Food and Agriculture Organization of the United Nations (FAO) / Geneva: World Health Organization (WHO), International Programme on Chemical Safety (IPCS). (Environmental Health Criteria, no 240) https://apps.who.int/iris/bitstream/handle/10665/44065/WHO_EHC_240_9_eng_Chapter6.pdf;jsessionid=423E9F9EF415CF750F5FF0200E5A22B6?sequence=9).

FSANZ (2021) Application A1210 Maltogenic alpha-amylase enzyme from GM *Saccharomyces cerevisiae*. Food Standards Australia New Zealand, Canberra https://www.foodstandards.gov.au/code/applications/Pages/A1210.aspx

Goesaert H, Leman P, Bijttebier A, Delcour JA. (2009) Antifirming effects of starch degrading enzymes in bread crumb. J Agric Food Chem 57:2346-2355.

IUBMB (2017) EC 3.2.1.133. https://www.qmul.ac.uk/sbcs/iubmb/enzyme/EC3/2/1/133.html accessed 24 March 2021

JECFA (2006) General specifications and considerations for enzyme preparations used in food processing. The Joint FAO/WHO Expert Committee on Food Additives http://www.fao.org/docrep/009/a0691e/A0691E03.htm

Poulsen LK (2004) Allergy Assessment of foods or ingredients derived from biotechnology, gene modified organisms, or novel foods. Mol Nutr Food Res 48:413-423.

Salkinoja-Salonen MS, Vuorio R, Andersson MA, Kämpfer P, Andersson MC, Honkanen-Buzalski T, Scoging AC (1999) Toxigenic strains of *Bacillus licheniformis* related to food poisoning. Appl Environ Microbiol 65:4637-4645

Schallmey M, Singh A, Ward OP (2004) Developments in the use of *Bacillus* species for industrial production. Can J Microbiol 50:1-17

USP (2018) Food Chemicals Codex 11th Edition, United States Pharmacopeial Convention, Rockville, Maryland