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Te Mana Kounga Kai – Ahitereiria me Aotearoa

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Supporting document

Risk and technical assessment – Application A1305

Alpha-amylase from *Bacillus licheniformis* (containing the gene for alpha-amylase from the gene variant ANZ105) as a processing aid

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Executive summary

Danisco Australia Pty Ltd has applied to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of the enzyme alpha-amylase (EC 3.2.1.1), from *Bacillus licheniformis* (*B. licheniformis*) containing the gene for alpha-amylase from the gene variant ANZ105 as a processing aid in starch processing to produce starch hydrolysates and the production of potable alcohol.

The available evidence provides adequate assurance that the proposed use of alpha-amylase from *B. licheniformis* as a processing aid is technologically justified. Alpha-amylase performs its technological function during food processing and, as such, meets the definition of a processing aid for the purposes of the Code. There are relevant identity and purity specifications for the enzyme in the Code and the applicant provided evidence that their enzyme preparation meets these specifications.

No public health or safety concerns were identified with the use of the production organism, which is neither pathogenic nor toxigenic. Analysis of the modified 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.

The alpha-amylase preparation is derived from the same safe strain lineage as an alpha-amylase produced by a *B. licheniformis* strain (JML-1584), previously reviewed by FSANZ as part of application A1219. The alpha-amylase from JML-1584 showed no evidence of genotoxicity *in vitro*. The no observed adverse effect level (NOAEL) in a 90-day oral gavage study in rats was 500 mg total organic solids (TOS)/kg bw/day.

The theoretical maximum daily intake (TMDI) of this alpha-amylase was calculated to be 1.15 mg TOS/kg bw/day. A comparison of the NOAEL and the TMDI results in a Margin of Exposure (MOE) of approximately 400.

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.

Overall, FSANZ concludes there are no safety concerns from the use of this alpha-amylase from *B. licheniformis* in the quantity and form required to perform its typical function in starch processing to produce starch hydrolysates and the production of potable alcohol, which must be consistent with Good Manufacturing Practice (GMP).

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1. Introduction

Danisco Australia Pty Ltd[1] has submitted an application to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of an alternative alpha-amylase (EC 3.2.1.1). This enzyme is produced by *Bacillus licheniformis* (*B. licheniformis)* containing the gene for alpha-amylase from the gene variant ANZ105.

The enzyme is proposed for use as a processing aid in carbohydrate processing to produce glucose syrups and other starch hydrolysates, and the production of potable alcohol. It will be utilised at the lowest effective level necessary to achieve the intended technological purpose, in accordance with Good Manufacturing Practice (GMP)[2].

Although the application refers to 'carbohydrate processing,' this report uses 'starch processing' as it is the established terminology in the Code for this type of processing aid. This reflects industry practice, where the terms are often used interchangeably in the beverage industry.

1.1 Objectives of the assessment

The objectives of this risk and technical assessment were to:

- determine whether the proposed purpose is solely technological, 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 food enzyme by considering the:
    - safety and history of use of the host organism
    - characterisation of the genetic modification(s) to the production strain
    - safety of the enzyme.

2 Food technology assessment

2.1 Identity of the enzyme

The applicant provided relevant information regarding the identity of the enzyme, and this has been verified using the IUBMB[3] enzyme nomenclature reference database (McDonald et al 2009). Details of the identity of the enzyme are provided below.

Accepted IUBMB name:	α-amylase
Systematic name:	4-α-D-glucan glucanohydrolase

[1] A subsidiary of International Flavors and Fragrances Inc (IFF)

[2] 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.

[3] International Union of Biochemistry and Molecular Biology.

Other names/common names:	Amylase, α- glycogenase; α amylase; endoamylase; Taka-amylase A; 1,4-α-D-glucan glucanohydrolase, Fortizyme, Buclamase
IUBMB enzyme nomenclature:	EC 3.2.1.1
CAS registry number:	9000-90-2

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.

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).

The alpha-amylase enzyme production will be produced according to GMP following standard food and feed enzyme manufacturing practices.

The alpha-amylase enzyme is produced through submerged fermentation with a *B. licheniformis* strain, using specified substrates and nutrients. After fermentation, the biomass is separated by centrifugation and filtration. The resulting fermentation broth containing the enzyme is further filtered and concentrated. The concentrated solution is then standardised and stabilised with diluents, followed by a final polish filtration.

Enzyme production is monitored and regulated through analytical and quality assurance procedures to confirm that the final product meets specifications and is suitable for use as a processing aid in food applications.

2.2.2 Specifications for identity and purity

There are international general specifications for enzyme preparations used in the production of food. These have been established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in its Compendium of Food Additive Specifications (FAO/WHO 2005) and in the Food Chemicals Codex (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 also includes specifications for arsenic and heavy metals (section S3—4) if they are not already detailed within specifications in sections S3—2 or S3—3.

The applicant submitted analytical results for three batches of their alpha-amylase enzyme preparation, all of which met international specifications established by JECFA and Food Chemicals Codex as well as those in the Code (as applicable), as shown in Table 1.

The specification for the enzyme preparation used by the manufacturer (as provided in section 2.5 of the application) includes a test for the absence of the production strain. The enzyme, however, is a biological isolate of variable composition, containing the enzyme

protein, as well as organic and inorganic material derived from the microorganism and fermentation process. Refer to Section 4 below for the total organic solids (TOS) value. TOS encompasses the enzyme component and other organic material originating from the production organism and the manufacturing process, while excluding intentionally added formulation ingredients.

Table 1 *Analysis of applicant's final 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)	<5	≤5	≤5	≤2
Arsenic (mg/kg)	<1	-	-	≤1
Cadmium (mg/kg)	<0.5	-	-	≤1
Mercury (mg/kg)	<0.5	-	-	≤1
Coliforms (cfu/g)	<30	≤30	≤30	-
***Salmonella* (in 25 g)**	Negative			-
***Escherichia coli* (in 25 g)**	Negative	Absent	-	-
Antimicrobial activity	Negative	Absent	-	-
Production strain	Negative			

cfu = colony forming units

2.3 Technological purpose and justification

The technological purpose of this alpha-amylase is use as a processing aid in the production of glucose syrups, other starch hydrolysates, and potable alcohol. This includes alcoholic beverages that have had the alcohol reduced or removed. This use is consistent with the typical function of alpha amylase and is supported by scientific literature, which indicates this enzyme is principally responsible for the technological purpose (Fox 2018, Balakrishnan, et al 2019). The applicant requested use of the enzyme at GMP levels.

As identified by the IUBMB (section 2.1, above), alpha-amylase catalyses the endohydrolysis of (1→4)-α-D-glucosidic linkages in polysaccharides containing three or more (1→4)-α-linked D-glucose units. For a schematic representation of the endohydrolysis reaction catalysed by alpha-amylase, refer to its record in the enzyme database BRENDA[4] .

2.3.1 Production of alcohol

Alpha-amylase is used to liquefy starch from sources like corn, wheat, barley, rye, triticale, added during the feed tank/mixing or secondary liquefaction step, in alcohol production. This improves extraction and saccharification to maximise fermentable carbohydrate yield and efficiently hydrolysing starch, increasing fermentable sugar content. After fermentation, solids and enzyme precipitates are separated from the slurry. The remaining liquid is distilled, and the alcohol is filtered through a high-temperature molecular sieve to remove residual water and water-soluble protein.

Technological benefits of alpha-amylase in this process include:

- effective starch liquefaction
- support for high adjunct use and energy efficiency

[4] EC explorer - BRENDA Enzyme Database (brenda-enzymes.org)

- rapid viscosity reduction
- flexibility in pH and temperature
- higher alcohol yields and reduced raw material usage due to improved processing.

2.3.2 Production of glucose syrups and other starch hydrolysates

Alpha-amylase is used to liquefy starch from various sources including wheat, barley and rice. The resulting glucose rich syrups can be further processed into dextrose, high fructose corn syrup, or fermented into organic acids, alcohol, or amino acids.

The technological benefits of using alpha-amylase in the production of glucose syrups and other starch hydrolysates are:

- efficient liquefaction of starchy substrates
- reduced viscosity of gelatinised starch
- increase in soluble dextrins and oligosaccharides
- improvement in process efficiency and product consistency
- minimisation of raw material consumption and waste.

The applicant submitted data regarding the physical and chemical characteristics of their enzyme preparation, as listed in Table 2. The enzyme is heat-denatured at 83°C after 30 minutes, resulting in its inactivation during the manufacturing processes of glucose syrups, starch hydrolysates, and potable alcohol. Consequently, the enzyme does not exert any technological effect in the final food product.

Table 2 *Alpha-amylase enzyme preparation physical/chemical properties*

Physical/chemical properties of commercial enzyme preparation	
Enzyme activity	Activity will depend on the final product. An example product has the Alpha-amylase activity range of 52515-64185 LPAU/g.
Appearance	The enzyme preparation presents as a liquid
Temperature range	Activity within range 30 -100°C Optimum 60-90°C
Temperature stability	Alpha-amylase is inactivated at temperatures above 83˚C when incubated for 30 minutes
pH range and optimum	Max activity @ 4 -7.5 Activity within range 3.5-9.2

*LPAU/g in the context of amylase activity stands for Liquid Pancreatic Amylase Units per gram is an industry-specific or proprietary unit used to quantify the activity of pancreatic amylase, particularly in liquid enzyme preparations

2.4 Allergen considerations

The applicant stated the enzyme preparation does not contain known food allergens. FSANZ has reviewed the information provided by the applicant that supports this assertion.

2.5 Food technology Conclusion

FSANZ concludes that the use of this alpha-amylase as a processing aid for use in the production of glucose syrups, other starch hydrolysates, and potable alcohol, including alcoholic beverages that have had the alcohol reduced or removed is consistent with its functions as a processing aid. 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.

Alpha-amylase performs its technological purpose during the production of the nominated foods, after which it is inactivated, and is not performing a technological purpose in the final food. It is therefore functioning as a processing aid for the purposes of the Code.

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

3 Safety assessment

The objective of this safety assessment is to evaluate any potential public health and safety concerns associated with the use of this alpha-amylase enzyme as a processing aid.

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

3.1 Source microorganism

B. licheniformis has a long history of safe industrial use, particularly in the production of enzymes for food processing, dating back to 1972 (De Boer et al. 1994; Sewalt et al. 2018; Muras et al. 2021). 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. It has numerous current and potential applications, 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 enzyme 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 confirmed the identity of the production organism *B. licheniformis*. Using the safe strain concept (Pariza and Johnson 2001), the risk of toxin production by the production organism was determined to be very low. Analysis of characteristics of three representative batches of enzyme, along with the described production methodology, demonstrated consistent and appropriate application of culture conditions across batches. Results confirmed the production organism is not detected in the final enzyme preparation.

FSANZ's microbiological assessment did not identify any public health and safety concerns related to the use of *B. licheniformis* as a production organism for alpha-amylase.

3.2 Characterisation of the genetic modification to the production organism

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

The gene encoding the alpha-amylase is an assembly of sequence from multiple bacterial alpha-amylase genes. Danisco has assigned this alpha-amylase gene variant the unique identifier of ANZ105. Data provided by Danisco and analysed by FSANZ confirmed the expected alpha-amylase amino acid sequence.

Expression cassettes containing the alpha-amylase gene were introduced into the genome of the host strain, *B. licheniformis* using standard molecular biology techniques. Native *B. licheniformis* genes were used as auxotrophic selection markers enabling the selection of positive transformants on minimal growth medium.

3.2.2 Characterisation of the inserted DNA

Data provided by Danisco and analysed by FSANZ confirmed the presence of the inserted DNA in the production strain. Antibiotic resistance genes were not introduced into the production strain.

3.2.3 Stability of the introduced DNA

The stability of the introduced DNA in the production strain was examined by genome sequencing. DNA was extracted and analysed from cultures at generation 0 and compared to the end of fermentation. No change was observed. The results substantiate the stability of the alpha-amylase gene in the production strain.

3.3 Safety of the enzyme

3.3.1 History of safe use

Alpha-amylases have a long history of safe use in food production. Several alpha-amylase preparations have been approved for use as processing aids by FSANZ and included in Section 18 of the Code, as part of applications A33, A467, A1185, A1195, A1210, A1211, A1219, A1231 and A1255 (FSANZ 2003, FSANZ 2020a, FSANZ 2020b, FSANZ 2021, FSANZ 2022a, FSANZ 2022b, FSANZ 2022c, FSANZ 2023)

3.3.2 Bioinformatic assessment of homology with known toxins

A bioinformatics search was performed by the applicant (August 2023) to compare the similarity of the alpha-amylase amino acid sequence to known toxins. The search was conducted using the UniProtKB database[5]. No matches of concern were identified in the assessment of homology with known toxins.

3.3.3 Toxicology data

Toxicity studies performed with an alpha-amylase produced by a *B. licheniformis* strain (JML-1584) in the same safe strain lineage as the production strain for the alpha-amylase that is the subject of this application. The alpha-amylase strain JML-1584 is produced by submerged fermentation of the *B. licheniformis* strain carrying the alpha-amylase gene originating from an isolate of *Cytophaga* species. This safe strain lineage concept is consistent with Food and Agriculture Organization/World Health Organization guidance on risk assessment of food enzymes (FAO/WHO 2020b).

Based on the available information, the test item used in the toxicity studies of alpha-amylase from JML-1584 is considered suitably equivalent for assessing the safety of the *B. licheniformis* production strain and the alpha-amylase enzyme concentrate that is the subject of this application.

Toxicological data for alpha-amylase from JML-1584 as an enzyme processing aid has previously been reviewed by FSANZ as part of application A1219, as available at the following link: A1219 - Alpha-amylase from GM Bacillus licheniformis as a processing aid | Food Standards Australia New Zealand (FSANZ, 2022c).

For A1219, the applicant submitted toxicological studies with their alpha-amylase enzyme preparation which were reviewed by FSANZ in the assessment:

- Bacterial reverse mutation assay[6]
- *In vitro* mammalian chromosomal aberration test[7]
- 13-week oral toxicity study in rats[8].

No public health and safety concerns were identified in the assessment of alpha-amylase from JML-1584 under the proposed use conditions in starch processing, brewing of beverages and production of potable alcohol. Toxicity testing of the alpha-amylase from JML-158 showed no evidence of mutagenicity, clastogenicity or aneugenicity *in vitro*. The no observed adverse effect level (NOAEL) in a 90-day oral gavage study in rats was the highest dose tested, 500 mg total organic solids (TOS)/kg bw/day.

[5] UniProt database
[6] Regulatory status: Good Laboratory Practice (GLP); conducted in accordance with Organisation for Economic Co-operation and Development (OECD) Test Guideline (TG) 471.
[7] Regulatory status: GLP; conducted in accordance with OECD TG 473.
[8] Regulatory status: GLP; conducted in accordance with OECD TG 408.

A review of all toxicological studies conducted with enzyme preparations produced by the applicant with their *B. licheniformis* strains further showed no evidence of genotoxicity or adverse effects on any specific target organ.

3.3.4 Potential for allergenicity

Searches were performed by the applicant (August 2023) to compare the similarity of the alpha-amylase amino acid sequence to known allergens. The searches were conducted using the Food Allergy Research and Resource Program (FARRP) AllergenOnline database[9] and the WHO/International Union of Immunological Societies (IUIS) Allergen Nomenclature database[10]. No matches to known allergens were identified in a search for >35% identity to known allergens in the alpha-amylase sequence using stretches of 80 amino acids or 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 the alpha-amylase preparation that is the subject of this application has not been evaluated by other regulatory agencies.

Alpha-amylase preparations from alternative production strains of *B. licheniformis* have been evaluated by the European Food Safety Authority (EFSA) (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) 2024, EFSA Panel on Food Enzymes (FEZ) 2025a, EFSA FEZ Panel 2025b). Alpha-amylase preparations from *B. licheniformis* have further been evaluated by Health Canada and approved for use under the List of Permitted Food Enzymes (Health Canada 2018). EFSA and Health Canada identified no public health and safety concerns associated with the intended conditions of use of alpha-amylase preparations from *B. licheniformis*.

Alpha-amylase preparations from *B. licheniformis*, including genetically modified strains, have been approved for use as processing aids in food in Denmark. Alpha-amylase from *B. licheniformis* is included in the positive list of approved processing aids in food in France.

The United States (US) Food and Drug Administration (FDA) has responded that it has ‘no questions’ to the GRAS notifications for alpha-amylase preparations from *B. licheniformis*, including alpha-amylase from JML-1584 for use as a processing aid in carbohydrate processing to produce sugar syrups and in fermentation to produce products (such as potable alcohol and organic acids) (FDA 1999a, FDA 1999b, FDA 2016a, FDA 2016b). GRAS notifications, and ‘no questions’ responses from the US FDA to GRAS notifications, do not constitute safety assessments by a national agency.

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 alpha-amylase enzyme preparation remained in the food.

The budget method is a valid screening tool for estimating the TMDI of a food additive (Douglass et al 1997). The calculation is based on physiological food and liquid

[9] AllergenOnline database
[10] WHO/IUIS Allergen Nomenclature database

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 (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 2020c). The method is used by overseas regulatory bodies and the FAO/WHO Joint Expert Committee on Food Additives (JECFA) (FAO/WHO 2021) for dietary exposure assessments for processing aids.

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

- the maximum physiological requirement for solid food (including milk) is 25 g/kg body weight/day
- 50% of solid food is processed
- all solid foods contain the highest use level of 15.84 mg TOS/kg in the raw material (starch for processing)
- modified starch in bakery and dairy products was the only use presented for solid food. Therefore, the enzyme preparation use level for this process of 0.79 mg TOS/kg final food was used in the budget method calculation for all processed solid food.
- 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)
- 25% of non-milk beverages are processed
- all non-milk beverages contain the highest use level of 15.84 mg TOS/kg in the raw material (starch for processing and cereal for the production of potable alcohol)
- among all non-milk beverages, potable alcohol produced the highest theoretical enzyme exposure when each application was assessed individually. Therefore, the enzyme maximal level in the final food of 45.30 mg TOS/kg food was used in the budget method calculation to represent all non-milk beverages.
- all of the TOS from the enzyme preparation remains in the final food.

The applicant provided further information as CCI about the assumptions behind the values used in their budget method calculation.

Based on these assumptions, the applicant calculated the TMDI of the TOS from the enzyme preparation to be 1.14 mg 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 body weight/day (the standard level used in a budget method calculation where there is potential for the enzyme preparation to be in baby foods or general-purpose foods that would be consumed by infants).
- FSANZ would 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 1.15 mg TOS/kg bw/day.

Both FSANZ and 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 and beverages whereas the applicant has stated that it is likely to be removed during production and refining processes. In addition, the enzyme would be denatured by heat after performing its technological function.

5 Discussion

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.

The alpha-amylase preparation is derived from the same safe strain lineage as an alpha-amylase produced by a *B. licheniformis* strain (JML-1584). Toxicological data for alpha-amylase from JML-1584 as an enzyme processing aid has previously been reviewed by FSANZ as part of application A1219.

Toxicity testing of the alpha-amylase from JML-1584 showed no evidence of mutagenicity, clastogenicity or aneugenicity *in vitro*. The no observed adverse effect level (NOAEL) in a 90-day oral gavage study in rats was 500 mg total organic solids (TOS)/kg bw/day, the highest dose tested. These findings confirm the safety of the *B. licheniformis* production strain and the alpha-amylase enzyme concentrate.

The TMDI was calculated by FSANZ to be 1.15 mg TOS/kg bw/day. A comparison of the NOAEL and the TMDI results in a Margin of Exposure (MOE) of approximately 400.

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

Overall, FSANZ concludes there are no safety concerns from the use of this alpha-amylase from *B. licheniformis* in the quantity and form required to perform its typical function in starch processing to produce starch hydrolysates and the production of potable alcohol, which must be consistent with GMP.

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