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

27 January 2021
148-21

Supporting document 1

Risk and technical assessment – Application A1210

Maltogenic alpha amylase enzyme from GM *Saccharomyces cerevisiae*

Executive summary

The purpose of the application is to amend Schedule 18 – Processing Aids of the Australia New Zealand Food Standards Code (the Code) to include a protein engineered maltogenic alpha-amylase (EC 3.2.1.133) from a genetically modified (GM) strain of *Saccharomyces cerevisiae.* The source organism for the enzyme gene is *Geobacillus stearothermophilus*. The proposed use of maltogenic alpha-amylase is as a processing aid in the manufacture of bakery products. It assists in limiting staling of baked products and so improves the quality and shelf life of the baked product, which is clearly articulated in the application.

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.

The safety 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 the production of food enzymes and raises no public health concerns. The GM production strain was confirmed to contain the inserted DNA and this DNA was shown to be inherited across several generations. While there is a lack of history of safe use of this specific enzyme, the alpha-amylase extracted directly from the source organism has a long history of safe use. The enzyme shows no significant homology to any known toxins. A degree of homology between the protein engineered maltogenic alpha-amylase and several respiratory allergens was found. However, respiratory allergens are generally not food allergens, and since the enzyme is completely degraded under the conditions of the human stomach, the risk of food allergy from the proposed uses of the enzyme is considered to be negligible.

Based on the reviewed toxicological data it is concluded that, in the absence of any identifiable hazard, an acceptable daily intake (ADI) ‘not specified’ is appropriate. A dietary exposure assessment was therefore not required.

Table of contents

1 Introduction

Lallemand Baking Solutions has applied to FSANZ, seeking permission for use of a new source of maltogenic alpha-amylase (EC 3.2.1.133) as a processing aid in baking. This enzyme is produced by a genetically modified strain of *Saccharomyces cerevisiae* expressing a protein engineered variant of the maltogenic alpha-amylase gene from *Geobacillus stearothermophilus*. The protein engineered variant confers improved thermostability of the enzyme for baking purposes.

The function of the enzyme is to catalyse the hydrolysis of starch polysaccharides in dough during the baking process, reducing crumb firmness and staling. If permitted following a pre-market assessment, the maltogenic alpha-amylase will provide an additional option for manufacturers of baked 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 *S. cerevisiae*. The donor microorganism of the maltogenic alpha amylase gene is *G. stearothermophilus* (see 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

[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):	80
Optimal pH:	5.5

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 yeast after completion of fermentation, autolysis of the yeast to release the enzyme, separation, purification and concentration of the enzyme using filtration processes and then formulation after spray drying using carriers such as maltodextrin to the appropriate product specification and packaging.

The manufacturing processes are such to ensure the production microorganism is removed from the final enzyme preparation. The source of the maltodextrin used as a carrier for the enzyme preparation is not sourced from wheat but from corn starch so it is not a potential allergen source. 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 three non-sequential batches 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). Analytical results for heavy metals (lead, arsenic, cadmium and mercury) confirm that representative batches meet the requirements of S3—4 of the Code.

[2] Chemical Abstracts Service

Table 2: Product specifications for commercial enzyme preparation

Analysis	Enzyme batch analysis	Specifications		
		JECFA	FCC[1]	Code
Lead (mg/kg)	<0.061, <0.250, <0.250	≤ 5	≤ 5	≤2
Arsenic (mg/kg)	<0.012, <0.025, 0.247	-	-	≤1
Cadmium (mg/kg)	<0.075, <0.050, <0.025	-	-	≤1
Mercury (mg/kg)	<0.025, <0.100, <0.050	-	-	≤1
Total coliforms (cfu/g)	0, 0, <10	≤30	≤30	-
Salmonella (in 25 g)	Absent	Absent	Negative	-
Enteropathic *E. coli* (in 25 g)	Absent	Absent	-	-
Antimicrobial activity	Absent	Absent	-	-
Production organism[2]	Absent	-	-	-
Recombinant DNA[2]	Absent	-	-	-

1. FCC – Food Chemical Codex; 2. Stated in the application to comply with European Food Safety Authority (EFSA) guidelines

The application provided analytical results indicating that representative samples of the commercial enzyme preparations do not contain any of the production source microorganism or recombinant DNA. This is indicated to comply with EFSA guidelines, but these are not official specification requirements.

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

The technological purpose of this enzyme is similar to that of other already permitted forms of the enzyme, in that it will be used in the manufacture of bakery products. Its purpose as a processing aid during the baking process is to reduce crumb firmness and staling in bread and other bakery products, thereby improving the quality and shelf life of these products.

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 the baking process 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.

2.4 Technological justification of the enzyme

Information was provided in the confidential commercial information section of the application supporting the benefits and technological justification of using the enzyme in the baking industry to reduce staling of the produced bread (or other bakery products). The details of a study assessing the impact on reducing staling of the produced bread using the applicant's enzyme compared to a control without using any enzyme and an alternative version of the enzyme were reviewed. The parameters assessed were crumb firmness and crumb resilience. The assessment concludes that both enzymes provided improved performance compared to the control produced without use of an enzyme. The results for all the different time points and the two parameters indicated the applicant's enzyme had comparable performance to the competitor's enzyme, some were slightly better and others slightly worse.

However after the full 2 weeks storage the applicant's performance was slightly better than the competitor's.

Ultimately it would be up to the end food producers to determine if the enzyme is of value to their production process and for their products. Various commercial considerations will also be important for any decisions.

2.3 Food technology conclusion

The proposed use is as a processing aid in the manufacture of bakery products to assist in limiting staling of the produced product and so improve the quality and shelf life of the baked product. FSANZ concludes that the evidence presented to support the proposed use 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

3.1 History of use

3.1.1 Host organism

Saccharomyces cerevisiae, also known as Baker's yeast, has a long history of use in food production. *S. cerevisiae* is a non-pathogenic and non-toxigenic species. The unmodified parent yeast strain is used in the commercial baking industry for bread production.

The production strain taxonomy has been confirmed as *S. cerevisiae* using whole genome sequencing (WGS) analysis. WGS was also used to confirm the absence of virulence factors, antimicrobial resistance genes and plasmids. Additional phenotypic AMR testing was performed to confirm the WGS findings using a set of four antibiotics: hygromycin, zeocin, geneticin and nourseothricin.

S. cerevisiae is listed as a source for an enzyme of microbiological origin (β-fructofuranosidase (EC 3.2.1.26)) in Schedule 18 – Processing aids of the Code. The *S. cerevisiae* production strain is maintained at the applicant's internal yeast culture collection.

3.1.2 Gene donor organism(s)

The maltogenic alpha-amylase gene was sourced from *Geobacillus stearothermophilus*, formerly known as *Bacillus stearothermophilus*. While *G. stearothermophilus* has been associated with food spoilage (André et al. 2017), it is not classified as a risk agent for human pathogenicity[3]. Furthermore, enzymes obtained from this organism have previously been approved and are listed in Schedule 18 of the Code, demonstrating a history of safe use for enzymes obtained from this microorganism.

[3] https://my.absa.org/Riskgroups

3.2 Characterisation of the genetic modification(s)

3.2.1 Description of DNA to be introduced and method of transformation

The gene that encodes the maltogenic alpha-amylase enzyme was chemically synthesised based on the sequence from *G. stearothermophilus.* The gene sequence has been codon-optimised to allow efficient expression in yeast. Modifications were also made to the amino acid sequence to improve thermostability. The expression cassette was generated with the enzyme gene flanked by specific promoters and terminators and was designed to allow targeted integration into the host genome. The transformation method was a standard method for the host species.

3.2.2 Characterisation of inserted DNA

A range of methods were used to characterise the insertion of the expression cassette. The data provided showed that the enzyme gene has been integrated into the targeted site, has the expected sequence and has not undergone rearrangement.

3.2.3 Genetic stability of the inserted gene

A genotypic analysis was performed in triplicate, comparing presence of the inserted DNA before and after a typical fermentation run. The data provided by the applicant shows that the expression of the gene is consistent across several generations, indicating the production strain is genetically stable.

3.3 Safety of maltogenic alpha amylase

The enzyme that is the subject of this application meets the specifications of JECFA and the Food Chemicals Codex.

3.3.1 History of safe use of the enzyme

Maltogenic alpha-amylase produced directly from the source organism, *G. stearothermophilus*, has been used since the mid-1990s in baking (Derde et al. 2012; Goesaert et al. 2009), and is approved for use in Australia, New Zealand and other countries. The maltogenic alpha-amylase that is the subject of this assessment is protein engineered and has not been the subject of assessment by any national or international regulatory agency. The US FDA responded with a “No Questions” letter to a GRAS Notification (GRN 842; January 2020) for this maltogenic alpha-amylase.

3.3.2 Bioinformatics concerning potential for toxicity

After generating a custom database of known toxins in UniProtKB[4] in November 2018, the applicant conducted a bioinformatics search using the amino acid sequence of this maltogenic alpha-amylase. There were no hits with a significant E-value, indicating a lack of similarity to any known toxin.

3.3.3 Stability of the enzyme in a simulated digestion assay

Simulated gastric digestion assay of the enzyme (unpublished study by Marzorati et al. 2020). Regulatory status: Not GLP

The test article for this assay was the enzyme that is the subject of the application. The

[4] https://www.uniprot.org/