Application to amend Schedule 18 of the Australian New Zealand Food Standards Code to include maltogenic α-amylase enzyme from a modified strain of *Saccharomyces cerevisiae* as a Processing Aid

Executive Summary

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This application dossier concerns the enzyme processing aid maltogenic α-amylase (4-α-D-glucan α-maltohydrolase; EC 3.2.1.133), produced by a genetically modified strain of *Saccharomyces cerevisiae* (traditional baker's yeast) that has been engineered to express an optimized variant of the maltogenic α-amylase gene from *Geobacillus stearothermophilus*..

The recipient *S. cerevisiae* strain has an extensive history of use in the food industry in the production of baked goods and in the production of food enzymes. It is recognised as a safe microorganism by various regulatory agencies worldwide. For example, it meets the required qualifications to be considered as a Qualified Presumption of Safety (QPS) organism by the European Food Safety Agency (EFSA) and is therefore presumed to be safe (EFSA BIOHAZ Panel, 2020).). Moreover, *S. cerevisiae* is an approved source organism for β-fructofuranosidase in the Australia New Zealand Food Standards Code, Schedule 18.

The maltogenic α-amylase donor organism *Geobacillus stearothermophilus* has a safe history of use in food and we were unable to identify any risk factors for using *G. stearothermophilus* as a gene donor. *Geobacillus stearothermophilus* is already listed as an accepted gene donor for both α-amylase and maltogenic α-amylase in the Australia New Zealand Food Standards Code, Schedule 18.

A whole genome sequencing of the production strain (source organism) has been performed, to characterize the strain and to demonstrate the absence of toxigenic potential and antibiotic resistance genes.
Also, the source organism has been determined to meet the safe strain criteria, based on the decision tree analysis developed by Pariza and Johnson (2001) for evaluating the safety of microbial enzymes.

The maltogenic α-amylase enzyme is produced from the *S. cerevisiae* production strain by fermentation, isolation and formulation. All the production steps are achieved in accordance with current good manufacturing practices (cGMP) and the principles of hazard analysis and critical control points (HACCP).

The enzyme is intended to be used as a technological aid in baking processes to reduce crumb firmness and staling in bread and other bakery products and is intended to substitute the use of other commercially available maltogenic α-amylase already evaluated and recognized as safe by various regulatory agencies and authoritative bodies all over the world.

The enzyme is added to the raw materials during the preparation of the dough and performs its technological function during baking. It is then expected to be inactivated and has no further technological effect after baking. The technological action of the enzyme processing aid is achieved by catalysing the hydrolysis of the starch polysaccharides in smaller molecules during baking. These molecules become too short to crystallise, and the formation of a permanent network is largely prevented leading to a reduction of bread staling.

The Total Maximum Daily Intake (TMDI) calculated for the maltogenic α-amylase enzyme processing aid using the Budget Method is 0.358 mg TOS/kg body weight per day based on the maximum intended level of use and the intended food uses. This TMDI, calculated using a conservative approach, is 889 times lower than the dose for which an adverse effect has been observed in an animal model with an equivalent maltogenic α-amylase (318.4 mg TOS/kg bw per day).

Based on the demonstration of the safety of the production strain and the absence of any hazards from the whole production process, we are confident that the maltogenic α-amylase enzyme processing aid from the modified strain of *saccharomyces cerevisiae* does not raise safety concerns for the intended use.

References

EFSA BIOHAZ Panel. Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA (2017-2019). *EFSA Journal* (2020a), 18(2):5966. Available at: https://doi.org/10.2903/j.efsa.2020.5966

Pariza M.W., Johnson E.A. . Evaluating the Safety of Microbial Enzyme Preparations Used in Food Processing: Update for a New Century. *Regulatory Toxicology and Pharmacology* (2001), 33(2), 173-186. Available at: https://doi.org/10.1006/rtph.2001.1466