Executive Summary

This application dossier concerns the enzyme processing aid lipase (EC 3.1.1.3), produced by a genetically modified strain of *Komagataella phaffii* (strain LALL-LI2) that has been engineered to express the native lipase gene from *Fusarium oxysporum*.

K. phaffii (formerly known as *P. Pastoris*) has an extensive history of use in the food and feed industry and has been utilized for many years for production of single-cell protein and enzymes (Spohner *et al*., 2015; Barone et al., 2023). It is recognised as a safe microorganism by various regulatory agencies worldwide. For example, *K. phaffii* is included in the list of organisms considered suitable for Qualified Presumption of Safety (QPS) approach for safety assessment by EFSA, with the qualification that it applies for production purposes and no viable cells are present in the final product (EFSA BIOHAZ Panel, 2024). Moreover, *K. phaffii* is an approved source for the production of soy leghemoglobin in the Australia New Zealand Food Standards Code, Schedule 26.

A synthetic DNA sequence of the lipase gene from *F. oxysporum* was used for the strain engineering. Therefore, no material from the donor organism was used in the construction of the modified yeast strain. Thus, the modified yeast contains only a limited sequence belonging to the gene of interest that was introduced to it, to produce lipase.
Fusarium oxysporum is already listed as an accepted gene donor for both lipase and trypsin 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 genes of potential concern.
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 lipase 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) for food and the principles of hazard analysis and critical control points (HACCP).

The enzyme is intended to be used in baking processes to improve dough structure and behavior during baking, increase bread volume and improve crumb structure and is intended to substitute the use of other commercially available lipase preparation 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, performs its technological function during dough handling, and is then denatured by heat during the baking step. It has no further technological effect after baking.

The Total Maximum Daily Intake (TMDI) calculated for the lipase enzyme processing aid using the Budget Method is 0.068 mg TOS/kg body weight per day based on the maximum intended level of use and the intended food uses.

Together, the totality of the available scientific data and information on the lipase food enzyme from *K. phaffii* LALL-LI2 indicates no safety concerns for the intended use.

References

EFSA BIOHAZ Panel. Update of the list of qualified presumption of safety (QPS) recommended microbiological agents intentionally added to food or feed as notified to EFSA 19: Suitability of taxonomic units notified to EFSA until September 2023. *EFSA Journal* (2024), 22(1): e8517. https://doi.org/10.2903/j.efsa.2024.8517

Pariza MW, Johnson EA. 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. https://doi.org/10.1006/rtph.2001.1466