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A Petition to Amend the Australia New Zealand Food Standards Code with a Lipase Enzyme Preparation produced by *Trichoderma reesei*

EXECUTIVE SUMMARY

The present application seeks to amend Standard 1.3.3. - Processing Aids of the Australia New Zealand Food Standards Code (the Code) to approve a triacylglycerol lipase enzyme preparation from *Trichoderma reesei* produced by AB Enzymes GmbH.

Proposed change to Standard 1.3.3 - Processing Aids

The table (section 1.3.3—6)—Enzymes of microbial origin in Schedule 18-4, is proposed to be amended to include a genetically modified strain of *Trichoderma reesei* as permitted source for triacylglycerol lipase (EC 3.1.1.3).

This application is submitted under a general assessment procedure.

Description of Enzyme Preparation

The food enzyme 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.

The main activity of the food enzyme is triacylglycerol lipase (EC 3.1.1.3).

Lipase hydrolyses ester bonds of triacylglycerols, resulting in the formation of mono- and diacylglycerols, free fatty acids and, in some cases, also glycerol. Lipases can be divided into four

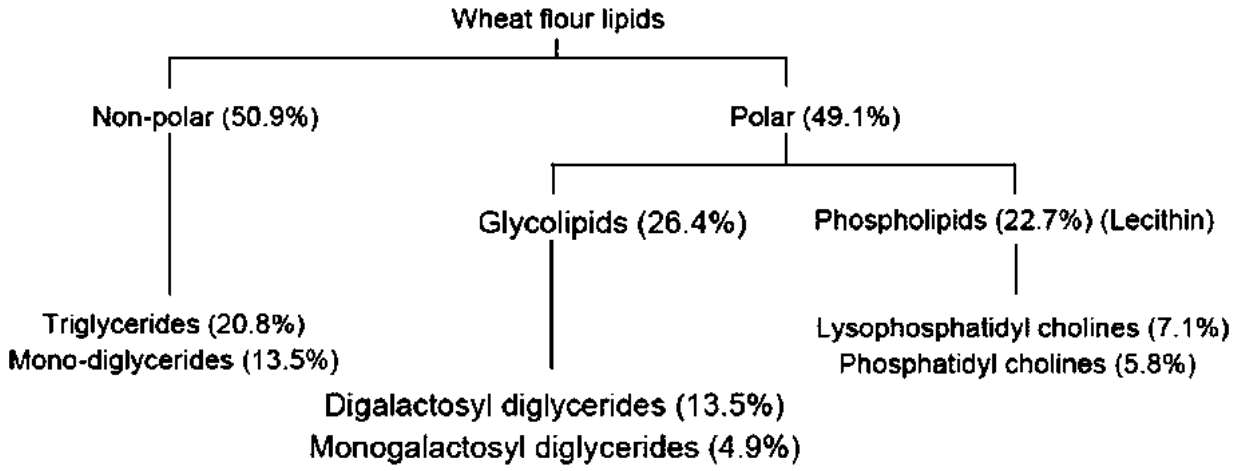
groups according to their specificity: substrate specific lipases, regioselective lipases, fatty acid specific lipases and stereospecific lipases.

The substrates for lipase are non-polar lipids such as triglycerides or triacylglycerol.

Triglycerides are formed by combining glycerol with three fatty acids molecules. The glycerol molecule has three hydroxyl (OH-) groups. Each fatty acid has a carboxyl group (COOH-). In triglycerides, the hydroxyl groups of the glycerol join the carboxyl groups of the fatty acid to form esters bonds:

$$HOCH_2CH(OH)CH_2OH + RCO_2H + R'CO_2H + R''CO_2H \rightarrow RCO_2CH_2CH(O_2CR')CH_2CO_2R'' + 3H_2O$$

Triglycerides are founds in plants and animals: they are the main constituents of vegetable oils and animal fats (Nelson et al., 2000). They are a major component of human skin oil (Lampe et al., 1983). Triglycerides and triacylglycerols are also found in wheat flour: wheat flour contains approximately 2.0–2.5% lipids; wheat lipids can be divided into polar (glycolipids, phospholipids) and non-polar lipids (triacylglycerides, mono-glycerides), as shown in the Figure 1 below:



It should be noted that the production organism is removed during filtration and is not present in the final enzyme preparation.

Use of the Enzyme

In general, the technological need of the enzymatic conversion of triglycerides with the help of triacylglycerol lipase can mainly be described as the degradation of a component (the substrate triglycerides).

Triacylglycerol lipase can be used in Cereal-based products (as of the Australian Food Code) such as, but not limited to, bread, biscuits, steamed bread, cakes, pancakes, tortillas, wafers and waffles. Non polar lipids such as triglycerides and triacylglycerols are found in wheat flour: wheat flour contains approximately 2.0–2.5% lipids; wheat lipids can be divided into polar (glycolipids, phospholipids) and non-polar lipids (triacylglycerides, mono-glycerides).

Food enzyme preparations are used by food manufacturers according to the Quantum Satis principle, which means that food manufacturers will typically fine-tune the enzyme dosage based on a dose range recommended by the enzyme supplier.

Benefits

Triacylglycerol lipase from *Fusarium sp.* expressed in *T.reesei* is mainly intended to be used in baking processes, (e.g. bread, biscuits, tortillas, cakes, steamed bread and croissants) and other cereal based processes (e.g. pastas, noodles and snacks).

The use of lipase can therefore influence the interactions between the different constituents of the dough, i.e. gluten proteins and lipids, starch and lipids as well as gluten and starch. The

benefits of the conversion of triglycerides (non-polar lipids) with the help of lipase in baking can therefore be summarized as follows:

- Facilitate the handling of the dough
- Improve dough stability and strength which results in processing tolerance
- Improve the dough's structure and behaviour during the baking steps
- Regulate batter viscosity, beneficial in the production process for e.g. waffles, pancakes and biscuits

The benefits of the conversion of the triglycerides (non-polar lipids) with the help of lipase in other cereal based processes can be summarized as follows:

- Facilitate the handling of the dough
- Improve dough stability and strength which results in processing tolerance
- Reduce oil uptake during frying

Safety Evaluation

The food enzyme object of the present dossier was subjected to several toxicological studies to confirm its safety for consumers. The mutagenicity studies showed that the food enzyme does not have the potential to damage the genetic material of living organisms, including mammals. The oral toxicity study showed that the food enzyme does not exhibit signs of toxicity, up to doses that are several thousand times higher than those which are consumed via food.

The product complies with the recommended purity specifications (microbiological and chemical requirements) of the FAO/WHO's Joint Expert Committee on Food Additives (JECFA) and the Food Chemicals Codex (FCC) for food-grade enzymes.

The product is free of production strain and recombinant DNA.

The safety of the Triacylglycerol lipase preparation was confirmed or is under consideration by external expert groups, as follows:

- **France**: The enzyme preparation was safety assessed according to the Guidelines for the evaluation of food enzymes. This resulted in the authorisation of the enzyme product by the French authorities in April 2017.
- **USA**: A GRAS no objection letter determined that the xylanase enzyme reparation is GRAS for its intended use GRAS #631
- **EFSA/ EU Commission**: A dossier was submitted in 2015 in compliance with Regulation (EC) 1332/2008 and is currently being reviewed by EFSA.

Conclusion

Based on the safety evaluation, AB Enzymes GmbH respectfully request the inclusion of triacylglycerol lipase from *Fusarium sp.* expressed in *T. reesei* in the table – section 1.3.3-6 - of schedule 18-4.; Permitted Enzymes of Microbial Enzymes.