AB Enzymes GmbH – Feldbergstrasse 78 , D-64293 Darmstadt

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

EXECUTIVE SUMMARY

The present application seeks to amend table to subsection S18—9(3), Permitted processing aids of various purposes of the Australia New Zealand Food Standards Code (the Code) to approve a lysophospholipase enzyme preparation from *Trichoderma reesei* produced by AB Enzymes GmbH.

Proposed change to Standard 1.3.3 - Processing Aids

The table schedule 18—9(3), Permitted processing aids various purposes, is proposed to be amended to include a genetically modified strain of *Trichoderma reesei* as permitted source for lysophospholipase (EC 3.1.1.5) for use in starch processing.

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 lysophospholipase (IUBM 3.1.1.5). The food enzyme catalyzes the hydrolysis of an ester bond between a fatty acid and glycerol in lysophospholipids, resulting in the formation of free fatty acids and glycero-phosphatide.

It uses lysophospholipids as substrates. Lysophospholipids are small (glycerol) phospholipids molecules, formed during the phospholipids breakdown as a result of the action of phospholipases. Although phospholipids are major component of all cell membranes in animals, plants and micro-organisms, lysophospholipids are found in only small amount in biological membranes. However, lysophospholipds and their receptors have been found in a wide range of tissues and cell types, indicating their importance in many physiological processes. Lysophospholipids are also known to be the predominant phospholipids found in wheat starch. Consequently, the substrate for lysophospholipase occurs naturally in nature (in particular in wheat based foods) and is therefore a natural part of the human diet.

Apart from lysophospholipase, the food enzyme also contains other enzymatic side activities in small amounts, which are naturally and typically produced by the production organism *Trichoderma reesei,* mainly xylanase, beta-glucanase and cellulase. However, these activities are not relevant from an application and/or safety point of view, due to small amounts and the fact that such enzyme activities have been used and approved for decades in food processing.

The production organism is removed during filtration and is not present in the final enzyme preparation.

Use of the Enzyme

In principle, the enzymatic conversion of conversion of lysophospholipids with the help of lysophospholipase can be used in the processing of all food raw materials which naturally contain lysophospholipids.

The food enzyme object of the dossier is typically used in starch processing, ie. such as the production of all kind of syrups (derived from wheat and corn/maize starches mainly).

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

This dossier is specifically submitted for the use of lysophospholipase **in starch processing, i.e. in the production of all kind of syrups** produced from starch, mainly wheat and maize/corn starches. Depending on the production process and the type of syrups to be produced, different enzymes are used (e.g. amylase, pullulanase…) to degrade starch.

Lysophospholipds present in starch (mainly wheat starch) can form micelles which negatively affect the filtration rate of the starch hydrolysates (syrups). In addition, they are known to form a complex with amylase, leading to a formation of a cloud in the final syrup, thus affecting its characteristics.

Therefore, the benefits of the conversion of lysophospholipids with the help of lysophospholipase are listed below:

- Prevent the formation of lysophospholipid micelles
- Facilitate the separation of undesired components
- Improve filtration rate (better and faster filtration)
- Improve the characteristics (clearness) of the filtrate
- Improve the environmental impact and sustainability (energy saving due to the load mitigation and decreased production time)

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, edition 10, 2016.

The product is free of production strain and recombinant DNA.

The safety of the *LPL* enzyme 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 July of 2013.
- **USA**: A GRAS determination was conducted and notified to the US FDA in May 2016 (GRN000653). In the reply letter from FDA, the agency had no questions regarding AB Enzymes' determination that the *LPL* enzyme reparation is GRAS for its intended use.
- **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 *Trichoderma reesei* expressing a lysophospholipase (*LPL*) gene from *Aspergillus nishimurae (ex*

A. fumigatus)[1] in the table to Schedule 18-9(3), standard 1.3.3.; Permitted processing aids various technological purposes.

[1] The strain was first identified as *Aspergillus fumigatus in 1999* and was recently identified by CBS as *Aspergillus nishimurae* within the section Fumigati of *Aspergillus*. As the name *Aspergillus fumigatus* has been used in our publications on lysophospholipase deriving from this strain, both names *Aspergillus fumigatus* and *Aspergillus nishimurae* are used for the donor organism.