

## Executive summary

The present application seeks to amend Schedule 18—Processing aids of the Australia New Zealand Food Standards Code (the Code) to approve a pullulanase enzyme preparation produced by Novozymes.

### Proposed change to Australia New Zealand Food Standards Code - Schedule 18—Processing aids

Schedule 18—Processing aids is proposed to be amended to include a genetically modified strain of *Bacillus subtilis* expressing a pullulanase from *Bacillus deramificans* as permitted source for pullulanase.

The application is applied for assessment by the general procedure.

### Description of enzyme preparation

The enzyme is a pullulanase (EC 3.2.1.41).

Pullulanase catalyses the hydrolysis of (1→6)- $\alpha$ -D-glucosidic linkages in pullulan, amylopectin and glycogen, and in the alpha- and beta-limit dextrins of amylopectin.

The enzyme is produced by submerged fermentation of a *Bacillus subtilis* microorganism expressing a pullulanase from *Bacillus deramificans*.

The pullulanase enzyme preparation is available as a liquid preparation complying with the JECFA recommended purity specifications for food-grade enzymes.

The producing microorganism, *Bacillus subtilis*, is absent from the commercial enzyme product.

### Use of the enzyme

The pullulanase enzyme preparation is used as a processing aid in starch processing for glucose syrups production and other starch hydrolysates. Generally, pullulanase hydrolyses 1,6- $\alpha$ -D-glucosidic linkages in pullulan and partially hydrolysed amylopectin as well as alpha- and beta-amylase limit dextrins of amylopectin<sup>1</sup>. When the substrate is partially hydrolysed amylopectin, linear maltodextrins like maltotriose and maltotetraose are released.

### Benefits

The benefits of the action of the pullulanase in starch processing for glucose syrups production and other starch hydrolysates are:

- Efficient degradation of starch increasing the substrate availability for other enzymes, thereby enabling higher yield of the substrate (dextrins) used for further processing and production of syrups.

---

<sup>1</sup> Pullulanase also hydrolyses 1,6- $\alpha$ -D-glucosidic linkages in glycogen, partially hydrolysed glycogen and glycogen limit dextrins. This is not known to have industrial food use

## Safety evaluation

The safety of the production organism and the enzyme product has been thoroughly assessed:

- The production organism has a long history of safe use as production strain for food-grade enzyme preparations and is known not to produce any toxic metabolites.
- The genetic modifications in the production organism are well-characterised and safe and the recombinant DNA is stably integrated into the production organism and unlikely to pose a safety concern.
- The enzyme preparation complies with international specifications ensuring absence of contamination by toxic substances or noxious microorganisms
- Sequence homology assessment to known allergens and toxins shows that oral intake of the pullulanase does not pose food allergenic or toxic concern.
- Two mutagenicity studies *in vitro* showed no evidence of genotoxic potential of the enzyme preparation.
- An oral feeding study in rats for 13-weeks showed that all dose levels were generally well tolerated and no evidence of toxicity.

Furthermore, the safety of the pullulanase preparation was confirmed by external expert groups, as follows:

- Denmark: The enzyme preparation was safety assessed resulting in the authorisation of the enzyme product by the Danish Veterinary and Food Administration.

## Conclusion

Based on the Novozymes safety evaluation, confirmed by the above-mentioned bodies, we respectfully request the inclusion of the pullulanase in Schedule 18—Processing aids.