

Carboxypeptidase from *Aspergillus oryzae*

**An application to amend the *Australia New Zealand Food Standards Code* with
a carboxypeptidase preparation produced by a genetically modified strain of
*Aspergillus oryzae***

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 carboxypeptidase enzyme preparation produced by Novozymes A/S.

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 *Aspergillus oryzae* expressing a carboxypeptidase from *Aspergillus oryzae* as permitted source for carboxypeptidase.

The application is applied for assessment by the general procedure.

Description of enzyme preparation

The enzyme is a carboxypeptidase D (EC 3.4.16.6), commonly known as carboxypeptidase.

Carboxypeptidases catalyse the hydrolysis of peptide bonds, preferably before C-terminal arginine and lysine residues.

The enzyme is produced by submerged fermentation of an *Aspergillus oryzae* microorganism expressing a carboxypeptidase from *Aspergillus oryzae*.

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

The producing microorganism, *Aspergillus oryzae*, is absent from the commercial enzyme product.

Use of the enzyme

The carboxypeptidase preparation is used as a processing aid in protein hydrolysis of peptide bonds in manufacturing and/or processing of proteins, yeast, and flavouring, the manufacture of bakery products and in brewing. Generally, carboxypeptidase degrade proteins into shorter proteins/peptides and free amino acids.

- In protein hydrolysis of peptide bonds in manufacturing and/or processing of proteins, yeast, and flavouring, the carboxypeptidase is used to hydrolyse proteins into shorter proteins/peptides and free amino acids.

- In the manufacture of bakery products, the carboxypeptidase partially hydrolyses the gluten network in the dough.
- In brewing, the carboxypeptidase increases the amount of free amino nitrogen by hydrolysis of proteins present in the raw materials.

Benefits

The benefits of the action of the carboxypeptidase in protein hydrolysis of peptide bonds in manufacturing and/or processing of proteins, yeast, and flavouring are:

- Use of more mild conditions than the alternative (acid hydrolysis at low pH and high temperature)
- Reduced formation of undesirable side-products (such as 3-monochloropropane-1,2-diol (3-MCPD)) compared with the alternative (acid hydrolysis at low pH and high temperature)
- Reduced formation of salt compared with the alternative (acid hydrolysis at low pH and high temperature)
- Formation of protein hydrolysates with a pleasant taste and minimal bitterness
- Improved protein solubility
- Formation of substances with a pleasant flavour

The benefits of the action of the carboxypeptidase in the manufacture of bakery products are:

- improved dough extensibility
- improved machinability in general and maintain the shape of laminated dough
- more uniform product, shape, surface and colour

The benefits of the action of the carboxypeptidase in brewing are:

- more uniform and predictable fermentation giving less variation caused by batch to batch variations of different raw materials like different malt batches
- increased flexibility in the choice of raw materials such as use of wheat, wheat malt, barley, corn and rice

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 carboxypeptidase 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.

Conclusion

Based on the Novozymes A/S safety evaluation, we respectfully request the inclusion of the carboxypeptidase in Schedule 18—Processing aids.