



**Petition to Amend Schedule 18 of the Australia New
Zealand Food Standards Code to Include
Protein-glutaminase from *Chryseobacterium proteolyticum*
as a Processing Aid**

- Executive Summary -

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Petition to Amend Schedule 18 of the Australia New Zealand Food Standards Code to Include Protein-glutaminase from *Chryseobacterium proteolyticum* as a Processing Aid

Executive Summary

Amano Enzyme Inc. (referred to Amano Enzyme hereafter) is proposing to amend Schedule 18 of the Australia New Zealand Food Standards Code to include protein-glutaminase from *Chryseobacterium proteolyticum* as an enzyme of microbial origin. Protein-glutaminase (EC 3.5.1.44, CAS number 62213-11-0) is an enzyme which catalyzes the deamidation of glutaminyl residues in the substrate polypeptide, resulting in the conversion of glutaminyl residues to glutamyl residues and release of ammonia. It is intended for use in various kinds of food processings to deamidate proteins in foods such as baking and pasta/noodle making, milk and dairy processing, meat and fish processing, grain processing and yeast processing. Protein-glutaminase is proposed for use as a processing aid in food productions at levels up to 0.64%.

The protein-glutaminase is an enzyme derived from non-genetically modified strain of *Chryseobacterium proteolyticum*. The production strain is obtained by several mutations from the original strain that was found Japanese soil. NTG (N-methyl-N'-nitro-N-nitrosoguanidine) was used to obtain the current production strain. The production process of the protein-glutaminase enzyme comprises a cultivation step with *Chryseobacterium proteolyticum*, followed by several filtration and purification steps to result in protein-glutaminase concentrate.

All of the raw materials used in the manufacture of the protein-glutaminase are safe and suitable for use. The enzyme is produced according to the FSSC22000 quality control system. Production controls are in place to monitor the strain during the fermentation and ensure the avoidance of genetic drift. Furthermore, the product specifications along with extensive batch analysis of protein-glutaminase demonstrate the purity of the enzyme preparation, including the absence of microbiological and heavy metal contaminants, as well as the lack of antibiotic activity.

Protein-glutaminase is stable at least 12 months from the manufacturing date under the sealed condition. The optimum pH range of Protein-glutaminase is 5.0 – 7.0 and the optimum temperature is 50 - 60°C. Protein-glutaminase is inactivated when exposed to temperature



greater than 70°C. Also, as far as Amano Enzyme is aware, Amano Enzyme's Protein-glutaminase described in this dossier does not have any enzymatic side activities which might cause adverse effect.

The safety of Protein-glutaminase derived from *Chryseobacterium proteolyticum* can be supported by its history of use, as well as toxicity studies. Protein-glutaminase has been approved by the following authorities:

- Protein-glutaminase complies with the internationally accepted JECFA specifications for chemical and microbiological purity of food enzymes (FAO/WHO, 2006).
- Protein-glutaminase from *Chryseobacterium proteolyticum* is approved in France (Arrêté du 19 octobre 2006).
- FDA responded to the GRAS notification submitted by the Amano Enzyme Inc. that FDA has no questions regarding that protein-glutaminase enzyme preparation from *Chryseobacterium proteolyticum* is GRAS (GRAS Notice No. GRN 267).

As for the toxicity studies, the food enzyme has been subjected to a standard package of toxicological tests, with the following results:

- Bacterial reverse mutation: No mutagenic activity under the given test conditions. (Hatano Research Institute, Food And Drug Safety Center, 2004)
- Chromosomal aberrations: No clastogenic activity under the given test conditions (Hatano Research Institute, Food And Drug Safety Center, 2004)
- Systemic toxicity: The No Observed Adverse Effect Level (NOAEL) is 46.4 mg TOS/kg bw/day, which is the middle dose in the study. (Bozo Research Center Inc. 2005)

Protein-glutaminase derived from *Chryseobacterium proteolyticum* also does not pose any allergenicity concerns, given the long history of use of the enzyme. Additionally, the homology search based on the allergen data base was conducted using the amino-acid sequence. As a result there was no match with any proteins caused for allergies.

Theoretical Maximum Daily Intake was calculated using the Budget Method. Based on this method, the Total TMDI of Protein-glutaminase was calculated as 0.38 mg TOS/kg bw/day. As described above, NOAEL of the enzyme is 46.4 mg TOS/kg bw/day. Consequently, the safety margin of Protein-glutaminase is 122 (46.4/0.38).

As such, no safety concerns are anticipated with the proposed use of Protein-glutaminase as a processing aid in Australia/New Zealand.

References

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