



## **Petition to Amend Schedule 18 of the Australia New Zealand Food Standards Code to Include Thermolysin (Protease) from *Geobacillus stearothermophilus* 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 Thermolysin (Protease) derived from *Geobacillus stearothermophilus* as an enzyme of microbial origin. Thermolysin (Protease) (EC 3.4.24.27, CAS number 9073-78-3) is an enzyme catalyzing the hydrolysis of proteins broad specificity and is intended for use in dairy processing, egg processing, meat and fish processing, protein processing, yeast processing and flavoring production. Thermolysin (Protease) is proposed for use as a processing aid in food productions at levels up to 0.24%. The effect of the enzymatic conversion with the help of Thermolysin (Protease) is the conversion of the substrate proteins and peptides in various proteinic food raw materials, which may result in improvement of organoleptic properties (taste and flavor), physiological properties (foamability, emulsifying ability, heat stability, viscosity) and nutritional properties (absorptivity, digestivity).

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

All of the raw materials used in the manufacture of the Thermolysin (Protease) 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 Thermolysin (Protease) demonstrate the purity of the enzyme preparation, including the absence of microbiological and heavy metal contaminants, as well as the lack of antibiotic activity.

Thermolysin (Protease) is stable at least 18 months from the manufacturing date under the



sealed condition. The optimum pH range of Thermolysin (Protease) is from 7 to 8.5 and the optimum temperature is 60-70°C. Thermolysin (Protease) is inactivated when exposed to temperature greater than 70°C. Also, as far as Amano Enzyme is aware, Amano Enzyme's Thermolysin (Protease) described in this dossier does not have any enzymatic side activities which might cause adverse effect.

The safety of Thermolysin (Protease) derived from *Geobacillus stearothermophilus* can be supported by its history of use, as well as toxicity studies. Thermolysin (Protease) has been approved by the following authorities:

- Protease is listed on the Food Additive Index of CODEX General Standard for Food Additives (GSFA) (INS: 1101(i)) (CODEX, 2015).
- Thermolysine from *Geobacillus stearothermophilus* is approved in France (AFSSA, 2006)
- Protease from *Geobacillus stearothermophilus* is approved in Denmark (DVFA, 2013).
- Protease from *Geobacillus stearothermophilus* is approved as a food additive in China (NHFPC, 2014).
- Protease, Bacterial (PC) from *Geobacillus stearothermophilus* is approved as a food additive in Korea (KFAC, 2015)
- Protease from *Geobacillus stearothermophilus* is on the "List of Existing Food Additives" published by the Ministry of Health and Welfare of Japan (MHLW, 2014).

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

- Micronucleus: No mutagenic activity under the given test conditions. (Nihon Bioresearch Center, Inc. 1985)
- Chromosomal aberrations: No clastogenic activity under the given test conditions (Bozo Research Center Inc. 2010)
- Systemic toxicity: The No Observed Adverse Effect Level (NOAEL) is 1,000 mg/kg bw/day (323.3mg TOS/kg bw/day), which is the high dose in the study. (RCC NOTOX.,1989)

Thermolysin (Protease) derived from *Geobacillus stearothermophilus* also does not pose any allergenicity concerns, given the long history of use of the enzyme. Additionally, the amino acid sequence of Thermolysin (Protease) does not indicate that Thermolysin (Protease) has a potency of any allergenicity concerns.



Theoretical Maximum Daily Intake was calculated using the Budget Method. Based on this method, the Total TMDI of Thermolysin (Protease) was calculated as 1.53 mg TOS/kg body weight/day. As described above, NOAEL of the enzyme is 323.3 mgTOS/kg/day. Consequently, the safety margin of Thermolysin (Protease) is 211 (323.3/1.53).

As such, no safety concerns are anticipated with the proposed use of Thermolysin (Protease) as a processing aid in Australia/New Zealand.

## References

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