Petition to Amend Schedule 18 of the Australia New Zealand Food Standards Code to Include Thermolysin (Protease) from *Anoxybacillus caldiproteolyticus* as a Processing Aid

- Executive Summary -

Prepared by: Amano Enzyme Inc.
1-2-7, Nishiki, Naka-ku,
Nagoya 460-8630
Japan

Submitted to: Food Standards Australia New Zealand
PO Box 7186
CANBERRA BC ACT 2610
AUSTRALIA

March 29, 2017

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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 *Anoxybacillus caldiproteolyticus* as an enzyme of microbial origin. Thermolysin (Protease) (EC 3.4.24.27, CAS number 9073-78-3) is a thermo-stable neutral metallo-proteinase and is an enzyme catalyzing the hydrolysis of peptide bonds. It is used as an enzyme for protein processing to improve physical properties in foods and is intended for use in various kind of foods such as 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 improve physical properties (foamability, emulsifying ability, heat stability, viscosity) and also improves organoleptic properties (taste and flavor) and nutritional properties (absorptivity, digestivity) in foods.

The Thermolysin (Protease) is an enzyme derived from non-genetically modified strain of *Anoxybacillus caldiproteolyticus*. 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 *Anoxybacillus caldiproteolyticus*, 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℃. 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 *Anoxybacillus caldiproteolyticus* can be supported by its history of use, as well as toxicity studies. Thermolysin (Protease) has been approved by the following authorities:

- *Geobacillus caldoproteolyticus* is the former name of *Anoxybacillus caldiproteolyticus*[1]. Protease from *Geobacillus caldoproteolyticus* (*Anoxybacillus caldiproteolyticus*) is approved in France (Arrêté du 19 octobre 2006).
- Protease from *Geobacillus caldoproteolyticus* (*Anoxybacillus caldiproteolyticus*) is listed in IPA Database by CCFA (Codex Committee on Food Additives).

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 *Anoxybacillus caldiproteolyticus* 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

[1] http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2016.4522/full#efs24522-bib-0021

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

AFSSA (2006). ARRETE Arrêté du 19 octobre 2006 relatif à l'emploi d'auxiliaires technologiques dans la fabrication de certaines denrées alimentaires. Available at: http://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000000271061&dateTexte=20151009

IPA Database by CCFA. Available at: http://ipa.ccfa.cc/substance?task=detail&substance_id=725

Nihon Bioresearch Center, Inc. (1985) [unpublished]. *Micronucleus Test of Thermolysin in Mice* [*Confidential*]. Prepared by Nihon Bioresearch Center, Inc., Gifu, Japan. October, 1985 (Study No.: 467).

Bozo Research Center Inc. (2010) [unpublished]. *Chromosomal Aberration Test in cultured Chinese Hamster Cells treated with Thermoase concentrate* [*Confidential*]. Prepared by Bozo Research Center Inc., Shizuoka, Japan. August 16, 2010 (Study No.: M-1426).

RCC NOTOX. (1989) [unpublished]. *91Day Oral Gavage Toxicity Study with Thermoase* [*Confidential*]. Prepared by RCC NOTOX., The Nether lands. October, 1989 (Study No.: RCC NOTOX 008696).