Petition to Amend Schedule 18 of the Australia New Zealand Food Standards Code to Include Triacylglycerol lipase from *Candida cylindracea* 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

April 27, 2016

Petition to Amend Schedule 18 of the Australia New Zealand Food Standards Code to Include Triacylglycerol lipase from Candida cylindracea 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 Triacylglycerol lipase derived from *Candida cylindracea* as an enzyme of microbial origin. Triacylglycerol lipase (EC 3.1.1.3, CAS number 9001-62-1) is an enzyme which hydrolyses lipids (triglycerides) into fatty acids and mono-, di-glycerides or finally glycerol. It is intended for use in baking, milk and dairy processing, and fats and oil processing. Triacylglycerol lipase is proposed for use as a processing aid in food productions at levels up to 0.082%. The effect of the enzymatic conversion with the help of Triacylglycerol lipase is the conversion of the substrate oil and fats in various food raw materials, which may result in improvement of organoleptic properties (flavor). Also, Triacylglycerol lipase can preferentially separate fatty acids besides EPA and DHA from oil to be able to produce high-content EPA and DHA oil.

The Triacylglycerol lipase is an enzyme derived from non-genetically modified strain of *Candida cylindracea*. The production strain is obtained by several mutations from the original strain that was found Japanese soil. NTG (N-methyl-N'-nitro-N-nitrosoguanidine), UV (Ultraviolet), EMS (Ethyl methanesulfonate) and CI (Cell isolation) were used to obtain the current production strain. The production process of the Triacylglycerol lipase enzyme comprises a cultivation step with *Candida cylindracea*, followed by several filtration and purification steps to result in Triacylglycerol lipase concentrate.

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

Triacylglycerol lipase is stable at least 18 months from the manufacturing date under the sealed condition. The optimum pH range of Triacylglycerol lipase is 7 and the optimum temperature is 40-50°C. Triacylglycerol lipase is inactivated when exposed to temperature

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

The safety of Triacylglycerol lipase derived from *Candida cylindracea* can be supported by its history of use, as well as toxicity studies. Triacylglycerol lipase has been approved by the following authorities:

- Lipase is listed on the Food Additive Index of CODEX General Standard for Food Additives (GSFA) (INS: 1104) (CODEX, 2015).
- Triacylglycerol lipase from *Candida cylindracea* is approved as a food additive in China (NHFPC, 2014).
- Triacylglycerol lipase from *Candida cylindracea* 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:

- Bacterial reverse mutation: No mutagenic activity under the given test conditions. (Bozo Research Center Inc. 2012)
- Chromosomal aberrations: No clastogenic activity under the given test conditions (Bozo Research Center Inc. 2012)
- Systemic toxicity: The No Observed Adverse Effect Level (NOAEL) is 10.2 g/kg bw/day (581 mg TOS/kg bw/day), which is the high dose in the study. (Bozo Research Center Inc. 2013)

Triacylglycerol lipase derived from *Candida cylindracea* 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 Triacylglycerol lipase was calculated as 0.102 mg TOS/kg body weight/day. As described above, NOAEL of the enzyme is 581 mg TOS/kg bw/day. Consequently, the safety margin of Triacylglycerol lipase is 5696 (581/0.102).

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

References

CODEX (2015). CODEX alimentarius. The 38th session of the Codex Alimentarius Commission (2015). Available at: http://www.fao.org/gsfaonline/additives/index.html

NHFPC (2014). List of Enzyme Preparation for Foods and Their Sources published by National Health and Family Planning Committee of China. Available at: http://gain.fas.usda.gov/Recent%20GAIN%20Publications/Standard%20for%20Food%20Additive%20Use%20-%20GB2760-2015_Beijing_China%20-%20Peoples%20Republic%20of_4-28-2015.pdf

MHLW (2014). List of existing food additives [complied and published by the Ministry of Health and Welfare on April 16, 1996]. Japan Food Chemical Research Foundation (JFCRF), Tokyo, Japan. http://www.ffcr.or.jp/zaidan/FFCRHOME.nsf/pages/list-exst.add [Effective from January 30, 2014, Last update: 06/30/2014].

Bozo Research Center Inc. (2012) [unpublished]. *A BACTERIAL REVERSE MUTATION TEST of Triacylglycerol lipase [Confidential].* Prepared by Bozo Research Center Inc., Shizuoka, Japan. December, 2012 (Study No.: T-1056).

Bozo Research Center Inc. (2012) [unpublished]. *Chromosomal Aberration Test in cultured Mammalian Cells treated with Triacylglycerol lipase concentrate* [*Confidential*]. Prepared by Bozo Research Center Inc., Shizuoka, Japan. November, 2012 (Study No.: T-G050).

Bozo Research Center Inc. (2013) [unpublished]. A 13-week *Oral Toxicity Study with Triacylglycerol lipase* [*Confidential*]. Prepared by Bozo Research Center Inc., Shizuoka, Japan. January, 2013 (Study No.: B-7279).