



Petition to Amend Schedule 18 of the Australia New Zealand Food Standards Code to Include Oryzin (Protease) from *Aspergillus melleus* 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 Oryzin (Protease) from *Aspergillus melleus* 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 Oryzin (Protease) derived from *Aspergillus melleus* as an enzyme of microbial origin. Oryzin (Protease) (EC 3.4.21.63, CAS number 9074-07-1) is an enzyme catalyzing the hydrolysis of proteins broad specificity and is intended for use in baking, dairy processing, egg processing, meat and fish processing, protein processing, yeast processing and flavoring production. Oryzin (Protease) is proposed for use as a processing aid in food productions at levels up to 0.14%. The effect of the enzymatic conversion with the help of Oryzin (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 Oryzin (Protease) is an enzyme derived from non-genetically modified strain of *Aspergillus melleus*. The production strain is obtained by several mutations from the original strain that was found Japanese soil. MSI (Mono Spore Isolation) and conventional mutagenesis using UV-irradiation, N-methyl-N'-nitrosoguanidine and ⁶⁰Co were used to obtain the current production strain. The production process of the Oryzin (Protease) enzyme comprises a cultivation step with *Aspergillus melleus*, followed by several filtration and purification steps to result in Oryzin (Protease) concentrate.

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



Oryzin (Protease) is stable at least 18 months from the manufacturing date under the sealed condition. The optimum pH range of Oryzin (Protease) is from 7 to 8 and the optimum temperature is 40°C. Oryzin (Protease) is inactivated when exposed to temperature greater than 55°C and a pH of lower than 4 or greater than 11. Also, as far as Amano Enzyme is aware, Amano Enzyme's Oryzin (Protease) described in this dossier does not have any enzymatic side activities which might cause adverse effect.

The safety of Oryzin (Protease) derived from *Aspergillus melleus* can be supported by its history of use, as well as toxicity studies. Oryzin (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).
- Protease (exopeptidase) from *Aspergillus melleus* is approved in France (AFSSA, 2006) and Denmark (DVFA, 2013).
- Protease from *Aspergillus melleus* is on the "List of Existing Food Additives" published by the Ministry of Health and Welfare of Japan (MHLW, 2014).
- Protease from *Aspergillus melleus* is approved as a food additive in China (NHFP, 2014).
- Protease from *Aspergillus melleus* is on a list of Permitted Food Enzymes in Canada (Health Canada, 2015).

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 (Amano Pharmaceutical Co., Ltd. 1993)
- Chromosomal aberrations: No clastogenic activity under the given test conditions (Amano Pharmaceutical Co., Ltd. 1994)
- Systemic toxicity: The No Observed Adverse Effect Level (NOAEL) is 1,356 mgTOS/kg/day, which is the high dose in the study. (Amano Pharmaceutical Co., Ltd. 1974)

Oryzin (Protease) derived from *Aspergillus melleus* also does not pose any allergenicity concerns, given the long history of use of the enzyme. Additionally, the amino acid sequence of Oryzin (Protease) does not indicate that Oryzin (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 Oryzin (Protease) was calculated as 1.725 mg TOS/kg body weight/day. As described above, NOAEL of the enzyme is 1,356 mgTOS/kg/day. Consequently, the safety margin of Oryzin (Protease) is 786 (1356/1.725).

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

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