Application for the Authorisation of an Invertase and Exo-Beta-Glucosidase Food Enzyme from *Aspergillus niger* Strain IN 319 in the European Union

Pursuant to

Regulation (EC) No 1332/2008 of the European Parliament and Council of 16 December 2008 on Food Enzyme

2.2 PUBLIC SUMMARY

NON-CONFIDENTIAL

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INTRODUCTION

Shin Nihon Chemical Co., Ltd (Shin Nihon) wishes to market a food enzyme containing invertase and exo-beta-glucosidase derived from a non-genetically modified strain of *Aspergillus niger*, designated as strain IN 319, as a food enzyme in the European Union (EU). The food enzyme is herein referred to as “the invertase and exo-beta-glucosidase food enzyme” or simply “the food enzyme”. The application is being made to allow the invertase and exo-beta-glucosidase food enzyme to be added to the Community list of food enzymes; two entries on the Community list of food enzymes, one for each catalytic activity, is requested.

TECHNICAL DATA

Identity of the Food Enzyme

The food enzyme subject of this application is a food enzyme containing invertase and exo-beta-glucosidase derived from a non-genetically modified strain of *Aspergillus niger*, designated as strain IN 319. The enzymes are identified by the following systematic names and numbers:

<u>Invertase</u>

Source (strain)	*Aspergillus niger* IN 319
Common/Accepted Name:	β-Fructofuranosidase
Shin Nihon Enzyme Name/Abbreviation:	INV
Other Names:	Invertase; saccharase; glucosucrase; β-h-fructosidase; β-fructosidase; sucrase; fructosyl invertase; acid invertase

Enzyme Classification Number of Enzyme Commission (EC) of the International Union of Biochemistry and Molecular Biology (IUBMB)]:	EC 3.2.1.26
Chemical/Systematic Name:	β-D-Fructofuranoside fructohydrolase
Chemical Abstracts Service (CAS) Number:	9001-57-4
European Inventory of Existing Chemical Substances (EINECS) Number or European List of Notified Chemical Substances (ELINCS) Number:	232-615-7

<u>Exo-beta-glucosidase</u>

Source (strain)	*Aspergillus niger* IN 319
Common/Accepted Name:	β-Glucosidase
Shin Nihon Enzyme Name/Abbreviation:	BGA
Other Names:	Gentiobiase; cellobiase; aryl-β-glucosidase; β- D-glucosidase; β-glucoside glucohydrolase; arbutinase; amygdalinase; *p*-nitrophenyl β-glucosidase; amygdalase; salicilinase
Enzyme Classification Number of Enzyme Commission (EC) of the International Union of Biochemistry and Molecular Biology (IUBMB)]:	EC 3.2.1.21
Chemical/Systematic Name:	β- D-Glucoside glucohydrolase
Chemical Abstracts Service (CAS) Number:	9001-22-3
European Inventory of Existing Chemical Substances Number (EINECS) or European List of Notified Chemical Substances Number (ELINCS):	232-589-7

Chemical Composition and Properties of the Food Enzyme

The invertase and exo-beta-glucosidase food enzyme produced with *A. niger* IN 319 is manufactured as an ultra-filtered concentrate that does not contain any added diluents and is characterised by its invertase and exo-beta-glucosidase activity. The enzymes are not modified by a post-translational process or by technological procedures, and are not protein engineered.

The food enzyme is routinely analysed to confirm the purity and absence of any external contaminants. The specifications established for the food enzyme comply with the current purity and microbial limits established for enzyme preparations by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and in the Food Chemicals Codex (FCC).

Invertase catalyses the hydrolysis of beta-fructosyl bonds occurring at the terminal non-reducing residues of beta-D-fructofuranosides (*e.g*., sucrose), releasing fructose. Exo-beta-glucosidase catalyses the hydrolysis of beta-glucosyl bonds occurring at the terminal non-reducing residues of beta-D-glucosides (*e.g.*, cellobiose, beta-D-glucans). The optimum temperature range and pH for the invertase and exo-beta-glucosidase activities of the food enzyme have been established experimentally. Both invertase activity and exo-beta-glucosidase activity become thermally unstable at high temperatures and are therefore heat-denatured (inactivated) during food processing at high temperatures. The shelf-lives of representative final formulated enzyme preparations have been established as 12 months when stored under the recommended conditions.

Source Materials and Manufacturing Process

Shin Nihon has established appropriate quality control procedures to ensure production of a high quality and safe food enzyme, including use of a safe production strain. The production strain from which the invertase and exo-beta-glucosidase food enzyme is produced is a non-genetically modified strain of the filamentous fungus *A. niger*, designated as strain IN 319. *A. niger* has an established history of safe commercial use in food production, as well as a history of safe use as a production organism used in the production of food enzymes globally. The production strain *A. niger* IN 319 is non-pathogenic and does not produce any known mycotoxins. The production strain has been deposited in a recognised culture collection and also is appropriately stored and monitored at Shin Nihon.

A Hazard Analysis and Critical Control Points (HACCP) plan is in place for the manufacture of the invertase and exo-beta-glucosidase food enzyme. The established quality control procedures ensure pure culture and optimum enzyme productivity conditions during fermentation. Manufacture of the food enzyme includes a purification process to ensure absence of microbiological contamination and removal of the production strain. Furthermore, only those batches of the food enzyme that meet the established specifications are released.

Reaction and Fate in Food

The invertase enzyme is intended for use in food processing in the hydrolysis of the beta-fructosyl bonds occurring at the terminal non-reducing residues of sucrose and other beta-D-fructofuranosides in various food and beverage matrices. The enzyme also catalyses fructotransferase reactions, a property used in the production of fructooligosaccharide (FOS) ingredients. The enzyme therefore performs its catalytic function directly on fructofuranoside (*e.g.*, sucrose) molecules present in food matrices during processing of the foods, releasing fructose. No safety concerns are raised with respect to the invertase reaction products as they consist of saccharides (*e.g.*, glucose, fructose, and FOS) that exist naturally in foods that are consumed by humans.

The exo-beta-glucosidase enzyme is intended for use in food processing in the hydrolysis of beta-glucosyl bonds occurring at the terminal non-reducing residues of beta-D-glucosides in various food and beverage matrices. The enzyme therefore performs its catalytic function directly on beta-D-glucoside molecules present in food matrices during processing of the foods, releasing glucose and aglycones. No safety concerns are raised with respect to the exo-beta-glucosidase reaction products (*i.e.*, glucose and aglycone compounds) as they consist of substances that exist naturally in foods that are consumed by humans.

Any residual invertase exo-beta-glucosidase following their addition during food processing will typically be heat-denatured; thus, the enzyme would have no technological effect on the final foods as consumed in most instances. The use of the standardised invertase enzyme preparations in the manufacture of some confectionary products (*e.g.*, cream centred chocolate candy), however, would not be followed by an inactivation or removal step, and therefore, the enzyme may continue to perform a technological function in the final food. Such uses of invertase, however, consist of well-established and typical applications in the food industry. Any residual exo-beta-glucosidase from such uses of standardised invertase enzyme preparations also would not be inactivated or removed, however, the enzyme would not perform a technological function in the final food due to the lack of substrate in these instances.

Proposed Conditions of Use in Food Manufacturing and the Proposed Maximum Use Levels

Following manufacture of the invertase and exo-beta-glucosidase food enzyme, the food enzyme is formulated into final enzyme preparations, which are standardised to either invertase or exo-beta-glucosidase activity.

The standardised invertase enzyme preparations are intended for use in food and beverage processing in the production of confectionery, fructose syrups, and particular spirits. The standardised invertase enzyme preparations also are intended for use in the production of food ingredients, namely FOS and flavourings. The ingredients in turn are used in a range of foodstuffs. FOS also is used in infant formulae and follow-on formulae. The primary technological need for invertase is for the conversion of sucrose to fructose and glucose for the purpose of improving taste, texture, and/or consistency of foods/beverages, as well as other desired effects, such as to improve preservation/shelf-life of fruit syrups, or to improve/accelerate fermentation during brewing. Invertase also catalyses fructotransferase reactions and is therefore applied in the production of FOS. Alternative procedures for the inversion of sucrose involve the use of acid hydrolysis; however, enzymatic inversion of sucrose provides an advantage over acid hydrolysis as the resulting syrup mixtures are free of coloured by-products that are produced during acid hydrolysis. The use of enzymes, such as invertase, by the food industry also has many other advantages over acid hydrolysis, including being higher yielding, and therefore, more cost efficient, more environmentally friendly producing less acidified water, and non-corrosive precluding the need to use corrosion-resistant materials.

The standardised exo-beta-glucosidase enzyme preparations are intended for use in the processing of fruit and vegetable products, tea-based beverages and alcoholic beverages. The standardised exo-beta-glucosidase enzyme preparations also are intended for use in the production of food ingredients, which are used in a range of foodstuffs. The primary technological need for exo-beta-glucosidase is for the hydrolysis of beta-glucosyl bonds of aroma and flavour precursors in various food matrices (*e.g.*, fruits, vegetables, tea leaves, and herbs) for the purpose of enhancing aroma and/or flavour of food/beverages and food ingredients.

The technological needs for invertase and exo-beta-glucosidase are established in the food industry and cannot be sufficiently met with the use of other enzymes or by other means.

The maximum use level of the food enzyme from the use of the standardised invertase enzyme preparations in the processing of the intended food and beverages is 38.5 mg TOS/kg of the final foodstuffs. The maximum use level of the food enzyme from the use of the standardised exo-beta-glucosidase enzyme preparations in final foods occurs in the processing of fruit/vegetable pastes and purees at 17.1 mg TOS/kg food, and during the processing of tea-based drinks and wines at 85.5 mg TOS/kg. The levels of the food enzyme in the final foodstuffs from the proposed uses in food ingredients does not exceed the maximum use levels used in the processing of foodstuffs as described above. FOS manufactured with invertase also is intended for use in infant and follow-on formulae[1]. The resulting maximum amount of the food enzyme potentially present in infant formula is 0.031 mg TOS/100 mL. The food enzyme is used only at the level required to achieve the intended effect.

The theoretical maximum daily intake (TMDI) calculated for the invertase and exo-beta-glucosidase food enzyme using Budget Method assumptions for exposure was 2.62 mg TOS/kg body weight/day from both solid foods and non-milk beverages. Intakes of the food enzyme from infant formulae and follow-on formulae among infants aged 0 to 12 months were calculated to be 0.05 mg TOS/kg body weight/day for infants less than 6 months and

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[1] In accordance with *Directive 2006/141/EC*

0.07 mg TOS/kg body weight/day for infants 6 to 12 months based on the uses of the food enzyme in the production of FOS.

TOXICOLOGICAL DATA

The core set of toxicological tests have been performed on Shin Nihon's the invertase and exo-beta-glucosidase food enzyme produced with *A. niger* IN 319 in accordance with EFSA's guidance on food enzyme dossiers. The food enzyme was non-mutagenic/non-genotoxic in the bacterial reverse mutation test and the *in vitro* chromosomal aberration test. In the repeated-dose 90-day oral toxicity study conducted in rats, the no-observed-adverse-effect level (NOAEL) for the oral toxicity of the invertase and exo-beta-glucosidase food enzyme in rats was determined to be 1,067 mg TOS/kg body weight/day, the highest dose tested. A large margin of safety exists between the NOAEL for the invertase and exo-beta-glucosidase food enzyme and the estimated maximum potential daily intakes on a TOS basis. Likewise, a large margin of safety exists between the NOAEL and the intakes from infant formulae and follow-on formulae among infants aged 0 to 12 months.

No concerns for allergenicity are raised as the potential for invertase and exo-beta-glucosidase produced by *A. niger* IN 319 to cross-react with known allergens is low. Additionally, there is no evidence from the available scientific literature or from the history of use of invertase and exo-beta-glucosidase enzyme preparations formulated with Shin Nihon's invertase and exo-beta-glucosidase food enzyme (produced with *A. niger* IN 319) in Japan indicating allergenicity to the enzyme in consumers. Based on this information, no evidence exists that might indicate that invertase or exo-beta-glucosidase produced by *A. niger* IN 319 would produce an allergenic response following use in food processing.