Application for the Authorisation of β-Fructofuranosidase from *Aspergillus fijiensis* ATCC® 20611™ as a Food Enzyme in the European Union

Under

Regulation (EC) No. 1331/2008 of the European Parliament and Council of 16 December 2008

PUBLIC SUMMARY

NON-CONFIDENTIAL

Amendment – Re-classification of Production Strain

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INTRODUCTION

BEGHIN MEIJI wishes to market β-fructofuranosidase from *Aspergillus fijiensis* ATCC® 20611™, as a food enzyme in the European Union (EU). The application is being made in order for the β-fructofuranosidase food enzyme to be listed on the Community list of food enzymes.

TECHNICAL DATA

Identity of the Food Enzyme

The food enzyme which is the subject of this application is β-fructofuranosidase derived from non-genetically modified *A. fijiensis* ATCC® 20611™. The enzyme is identified by the following systematic names and numbers:

Common or Usual Name:	β–fructofuranosidase
Trade Name:	Fructosylfuranosidase
Chemical Name:	β-D-fructofuranoside fructohydrolase
Synonyms:	Invertase
International Union of Biochemistry and Molecular Biology (IUBMB) Enzyme Nomenclature:	β-fructofuranosidase
IUBMB Number: [Enzyme Commission (EC) Number]	EC 3.2.1.26
Chemical Abstracts Service (CAS) Number:	9001-57-4
European Inventory of Existing Chemical Substances Number (EINECS):	232-615-7
European List of Notified Chemical Substances Number (ELINCS):	Not available

Chemical Composition and Properties of the Food Enzyme

The β–fructofuranosidase food enzyme is manufactured using a non-genetically modified, proprietary strain of *Aspergillus.* The majority of the enzyme preparation is composed of protein and carbohydrate, with the remainder of the product consisting of moisture, fat and ash. Sodium benzoate is added during manufacturing as a preservative. The enzyme is not modified by a post-translational process or by technological procedures, and is not protein engineered.

Every batch of the food enzyme is analysed to confirm the purity of the final material and absence of any external contaminants. The specifications established for the enzyme preparation 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), with additional internal stringent specifications.

The main enzymatic activity of this food enzyme is fructose-transferring activity, which transforms sucrose as a substrate to fructooligosaccharides (FOS), with simple sugars produced as a by-product. There are negligible levels of other enzymatic activities detected in samples of β–fructofuranosidase . Data is available in the literature regarding the optimal conditions for the activity of this enzyme. The recommended shelf-life for β-fructofuranosidase is 18 months, at temperatures at or below 25°C.

Source Materials and Manufacturing Process

The production organism is a proprietary micro-organism. There have been contradicting findings regarding ochratoxin A production among a number of strains of *A. japonicus*[1]. On this basis, every batch of the enzyme preparation is tested for a range of mycotoxins, including ochratoxin A. The production microorganism is maintained in carefully controlled conditions to ensure the consistency of the strain.

β-Fructofuranosidase is derived from the fermentation of a non-genetically modified strain of *A. fijiensis* ATCC® 20611™. The manufacturing process involves a series of fermentation stages followed by a number of purification steps. All raw materials and processing aids are compliant with Japanese Pharmacopoeia and Food Law. Furthermore, the enzyme is manufactured in accordance with Hazard Analysis Critical Control Point (HACCP) principles. Finally, only those batches of the food enzyme that meet the established specifications are released.

[1] The results of a comprehensive literature search have not identified any studies addressing the pathogenic or toxigenic potential of *A. fijiensis*, therefore the information relating to *A. japonicus* (previous classification) is included.

Reaction and Fate in Food

β-Fructofuranosidase is intended for use in the manufacture of FOS from sucrose. The β-fructofuranosidase is reacted with the sucrose substrate for several hours under conditions in line with those suitable for the food enzyme. Simple sugars and FOS are produced as a result of this reaction; the simple sugars are removed. This FOS can then be dried and packaged for further processing.

The enzymatic reaction between the food enzyme and the substrate occurs early in the FOS manufacturing process. This reaction is followed by a series of stages which either inactivate or remove the food enzyme from the FOS material, reducing possible adverse effects on nutrients or any other components of the food.

Case of Need and Proposed Conditions of Use

β-Fructofuranosidase is intended for use as a processing aid in the production of FOS using sucrose as a substrate. The final FOS material is used as an ingredient in a range of products for the purposes of fibre enrichment and/or sugar reduction.

The maximum potential use level of food enzyme in FOS has been calculated at 27.73 mg TOS/kg on the ingredient. The maximum potential use level of FOS in any of the intended final food uses is 50%. FOS is also permitted for use in infant and follow-on formulae in accordance with Directive 2006/141/EC.

Dietary Exposure

The theoretical maximum daily intake (TMDI), or maximum potential exposure to the food enzyme based on the proposed uses of FOS in foods, calculated for adults was 0.52 mg TOS/kg body weight/day. For infants and young children, exposure was calculated using both budget method assumptions or considering formulae as the sole source of energy in the diet; intakes ranged from 0.02 to 0.03 mg TOS/kg body weight/day.

Information on Existing Authorisations and Evaluations

The use of this food enzyme in food processing, namely FOS production, has been assessed by a number of international competent authorities. The enzyme has a long history of use, with the first approval published by the Conseil Supérieur d'Hygiène Publique de France (CSPH) in 1990. Since then, it has been approved for use by Food Standards Australia New Zealand (FSANZ), the United States Food and Drug Administration (U.S. FDA), Health Canada and the Japanese Ministry of Health, Labour, Welfare.

Toxicological Data

The full set of toxicological tests was performed using a representative batch of β-fructofuranosidase. The toxicological tests consisted of 2 *in vitro* genotoxicity tests, including a bacterial reverse mutation test and an *in vitro* micronucleus assay, and a 90-day

oral toxicity study conducted in rats. The food enzyme was demonstrated not to be mutagenic or genotoxic based on the *in vitro* genotoxicity tests. Based on the findings of the oral toxicity study, a no-observed-adverse-effect level (NOAEL) of 1,000 mg/kg body weight/day (the highest dose tested) equivalent to 920 mg/kg body weight/day when expressed as TOS was determined. There was therefore a considerable margin of exposure between this level and the TMDI calculated in the exposure assessment calculations.

No issues were identified in relation to the allergenicity of the food enzyme. Furthermore, a number of steps are incorporated into the FOS manufacturing process to ensure inactivation and removal of the enzyme from the final ingredient. The potential for an allergenic reaction is thereby further reduced.