APPLICATION FOR THE AUTHORIZATION OF FRUCTOSYLTRANSFERASE FROM *ASPERGILLUS ORYZAE* IN AUSTRALIA AND NEW ZEALAND

Executive Summary

PREPARED FOR:
Standards Management Officer
Food Standards Australia New Zealand
Ground Floor, Boeing House
55 Blackall Street
Barton ACT 2600
Australia

PREPARED BY:
Tate & Lyle Solutions USA LLC
5450 Prairie Stone Pkwy,
Hoffmann Estates, IL, 60192
USA

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Tate & Lyle Solutions USA LLC ("Tate & Lyle") is submitting an application for the authorisation and inclusion of fructosyltransferase (EC 2.4.1.9) derived from non-genetically modified *Aspergillus oryzae* in Standard 1.3.3 of the Food Standards Code ("the Code"). The food enzyme is intended for use as a processing aid in the production of short-chain fructooligosaccharides (sc-FOS) from sucrose. A related enzyme, β-fructofuranosidase (EC 3.2.1.26), derived from *Aspergillus niger*, *Aspergillus fijiensis*, and *Trichoderma reesei* is currently approved for use in Australia and New Zealand as per Schedule 18. The enzyme utilizes both invertase/β-fructofuranosidase and fructosyltransferase activities to produce sc-FOS. The primary activity of Tate & Lyle's enzyme is fructosyltransferase activity.

Tate & Lyle's fructosyltransferase is manufactured in accordance with current Good Manufacturing Practice (cGMP) and Food Safety System Certification (FSSC) 22000. The food enzyme is produced using food-grade materials and using quality-controlled fermentation and purification/recovery processes. The production strain has been deposited in a recognised culture collection. The strain was isolated from a food source and was selected as the production strain based on its capacity to produce high levels of fructosyltransferase activity, its cell viability, and its suitability for the industrial production of food enzymes, owing to its lack of pathogenicity and toxigenicity. The production organism, *A. oryzae*, is well characterized and has a long history of safe use in food production and the production of enzymes used in food processing, and there have not been any known reports of pathogenicity and/or virulence.

The fructosyltransferase food enzyme produced from *A. oryzae* is manufactured as a liquid concentrate that is then immobilized onto food-grade resin. The product specifications are compliant with the purity requirements for enzyme preparations established by the Food Chemicals Codex and the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Analytical data on 3 non-consecutive production-scale batches of the immobilised fructosyltransferase food enzyme demonstrate that the manufacturing process produces a consistent product that conforms to the product specifications. In addition, the same production batches were confirmed to be absent of mycotoxins and secondary metabolites.

The fructosyltransferase food enzyme, also referred to as β-fructofuranosidase or invertase, derived from various sources including *A. niger*, *A. fijiensis*, *A. oryzae*, *Trichoderma reesei, Saccharomyces* sp., and *Penicillium roqueforti* are approved for use in Australia and New Zealand, Canada, China, France, and Japan. In the United States (U.S.), the Generally Recognized as Safe (GRAS) status of an invertase enzyme from *Saccharomyces cerevisiae* intended for general use in food at cGMP was notified to the U.S. FDA and received "no questions." In Japan, the β-fructofuranosidase food enzyme is currently classified as a food additive and is permitted for use under the List of Existing Food Additives, Item No. 33: Invertase, with no official specification established under the 8th Japanese Specifications and Standards of Food Additives. The 9th Japanese Specifications and Standards of Food Additives, however, reclassified the enzyme as "fructosyl transferase," establishing it as an enzyme that transfers the fructosyl group of sugars and is obtained from the mould cultures *Aspergillus genus* and *Penicillium roqueforti*. In the European Union, one application for the authorisation of the β-fructofuranosidase food enzyme from *Saccharomyces cerevisiae* strain INV has been submitted to the European Commission and received a positive opinion by EFSA CEP Panel.

The fructosyltransferase food enzyme catalyses the transfer of fructose units to sucrose or other fructans, resulting in the production of sc-FOS such as 1-kestose, nystose, fructosyl-nystose, in addition to glucose. When the substrate is sucrose, it is first hydrolysed to glucose and fructose. This hydrolytic (fructofuranosidase) activity is a minor activity of the enzyme that is essential for the generation of sc-FOS.

The dietary exposure to the enzyme was not estimated based on analytical data demonstrating the absence of enzyme total organic solids in the final sc-FOS product.

The potential toxicity of the fructosyltransferase enzyme was addressed based on the established history of consumption of the food enzyme, the lack of similarity of the amino acid sequence to known protein toxins, and the extensive digestion of the enzyme predicted in digestion models. Toxicological studies were not conducted using the fructosyltransferase enzyme from *A. oryzae* as dietary exposure to the immobilised food enzyme is not expected. Toxicological evaluations of fructosyltransferase/*beta*-fructofuranosidase enzymes from other species, *Aspergillus brunneoviolaceus* and *Aspergillus fijiensis* strain ATCC 20611, published in the literature or reviewed as part of an application to FSANZ corroborate the safety Tate & Lyle's fructosyltransferase. The results of the genotoxicity studies indicated that the enzymes do not pose mutagenic or clastogenic concerns. The NOAELs from 90-day oral toxicity studies using *beta*-fructofuranosidase from *A. brunneoviolaceus* and *beta*-fructofuranosidase from *A. fijiensis* strain ATCC 20611 were concluded to be 1,200 mg TOS/kg body weight/day and 920 mg TOS/kg body weight/day, respectively, the highest doses tested in each study.

The potential allergenicity of the fructosyltransferase from *Aspergillus oryzae* was assessed by investigating sequence homology between the enzyme and known food allergens using the methodology described by FAO/WHO, Codex Alimentarius, and EFSA GMO Panel. No significant results suggestive of allergenic cross-reactivity of the fructosyltransferase to known allergens were identified. No scientific reports that suggest *A. oryzae* would induce an allergic response following consumption were identified. Considering that the food enzyme would be denatured if carried over into the final sc-FOS product under the conditions of food processing, it is not expected to pose an allergic risk to consumers.

The totality of the data provided in this application supports the conclusion that Tate & Lyle's fructosyltransferase from non-genetically modified *A. oryzae* is safe for use as a processing aid in the production of sc-FOS and does not present a risk to human health. The food enzyme is compliant with the purity requirements for enzyme preparations established by the Food Chemicals Codex and JECFA. The production organism is non-toxic, non-pathogenic, and is suitable for use in food production. Therefore, the authorisation of fructosyltransferase from a non-genetically modified source of *Aspergillus oryzae* in Australia and New Zealand does not present a safety concern.