FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

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Supporting Document

Risk and technical assessment – A1299 - fructosyltransferase from *Aspergillus oryzae* as a processing aid.

Executive summary

Food Standards Australia New Zealand (FSANZ) received an application from Tate and Lyle Solutions USA LLC to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of immobilised fructosyltransferase as a processing aid in the manufacture of short-chain fructooligosaccharides (sc-FOS) from sucrose.

The proposed use of this immobilised fructosyltransferase as an enzyme processing aid in the quantity and form proposed to be used is consistent with its typical function. Immobilised fructosyltransferase performs its technological purpose during processing of food and does not perform its technological purpose in the food for sale, therefore functioning as a processing aid for the purposes of the Code.

The enzyme preparation meets relevant identity and purity specifications.

The microbiological assessment undertaken by FSANZ did not identify any public health and safety concerns associated with the use of *A. oryzae* as a source of fructosyltransferase.

The fructosyltransferase has limited potential for dietary exposure when used as immobilised fructosyltransferase for sc-FOS production and there are no toxicity concerns associated with the enzyme preparation or the immobilisation resin.

There is no need to establish an ADI on the basis that there is only contact with the enzyme preparation during food processing, and the likelihood of residues in the final food is negligible. As such, dietary exposure modelling was not needed for the safety assessment. The enzyme preparation is not expected to pose a food allergenicity concern under the proposed conditions of use.

Overall, FSANZ concludes there are no safety concerns from the use of immobilised fructosyltransferase derived from *A. oryzae* in the quantity and form consistent with its typical function in the manufacture of sc-FOS from sucrose.

Table of contents

1. Introduction

Tate and Lyle Solutions USA LLC (Tate and Lyle) applied to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of the enzyme fructosyltransferase (EC 2.4.1.9) sourced from non - genetically modified *Aspergillus oryzae.*

The enzyme preparation is intended to be used as a processing aid in the manufacture of short-chain fructooligosaccharides (sc-FOS) from sucrose, at the minimum level required to achieve the desired effect, in accordance with the principles of Good Manufacturing Practice (GMP).

The objectives of this risk and technical assessment were to:

- determine whether the proposed purpose is solely technological, and that the enzyme preparation achieves its technological purpose as a processing aid in the quantity and form proposed to be used.
- evaluate potential public health and safety concerns that may arise from the use of this food enzyme preparation by considering the safety and history of use of the production organism and the safety of the enzyme.

Some information relevant to this assessment is commercially confidential information (CCI), therefore some details cannot be provided in a public report.

2. Food technology assessment

2.1. Identity of the enzyme

The enzyme fructosyltransferase (EC 2.4.1.9) is sourced from *A. oryzae*. The enzyme is used to produce sc-FOS from sucrose, the first step being a hydrolytic mechanism that splits the sucrose and then a fructosyl transfer mechanism to initiate production and elongation of sc-FOS molecules. The applicant's enzyme preparation is manufactured as a liquid concentrate that is then immobilised on to a food-grade ion-exchange resin before use.

The application refers to a related enzyme, β -fructofuranosidase (EC 3.2.1.26). However, the applicant confirmed that while their fructosyltransferase has both fructosyltransferase and fructofuranosidase activities, the primary activity of the applicant's fructosyltransferase is fructosyltransferase activity.

2.1.1. Fructosyltransferase (EC 2.4.1.9)

Fructosyltransferase is an enzyme that catalyses the transfer of a fructosyl group from one molecule to another, forming a fructosylated product (Table 1; Figure 1).

The applicant provided relevant information regarding the identity of immobilised fructosyltransferase. The identity of the fructosyltransferase was confirmed using ExplorEnz[1], the IUBMB Enzyme nomenclature and classification list.

Table 1 Sucrose:(2→1)-β-D-fructan 1-β-D-fructosyltransferase

Parameter	**Enzyme**
Accepted IUBMB name:	inulosucrase
Systematic name:	sucrose:(2→1)-β-D-fructan 1-β-D-fructosyltransferase
Other names/common names	**fructosyltransferase**, sucrose 1-fructosyltransferase; sucrose:2,1-β-D-fructan 1-β-D-fructosyltransferase
IUBMB enzyme nomenclature	EC 2.4.1.9
ECTree	2 Transferases 2.4 Glycosyltransferases 2.4.1 Hexosyltransferases 2.4.1.9 inulosucrase
CAS number:	9030-16-4
Reaction:	sucrose + $[(2\rightarrow1)$-β-D-fructosyl$]_n$ = glucose + $[(2\rightarrow1)$-β-D-fructosyl$]_{n+1}$.
Comments	Converts sucrose into inulin and D-glucose. Some other sugars can function as D-fructosyl acceptors.

[1] ExplorEnz: Official IUBMB Enzyme List (enzyme-database.org)

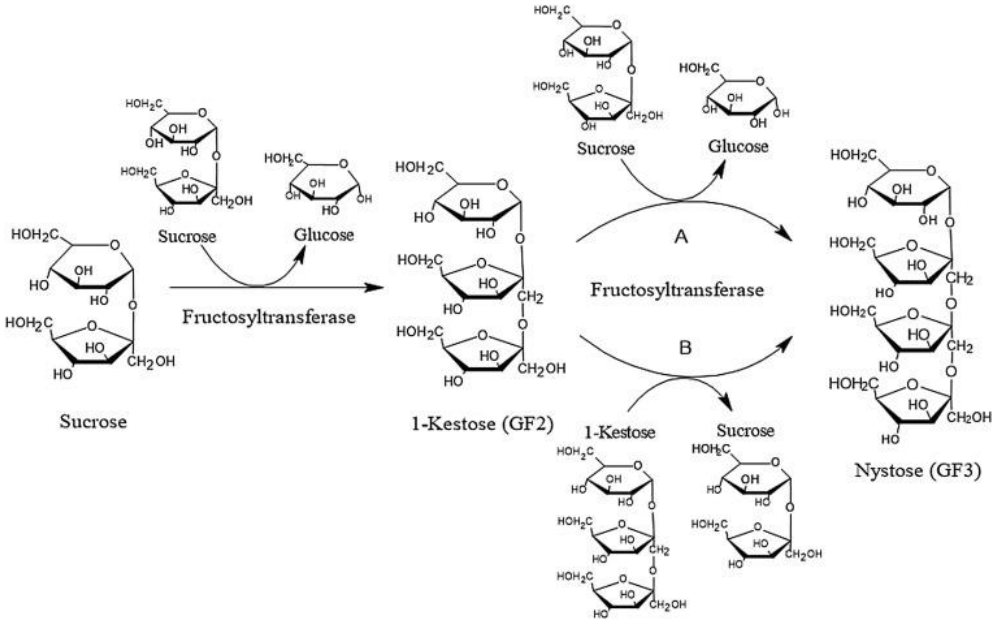

Figure 1 Enzymatic reaction of fructosyltransferase catalysing the fructosyl transfer from sucrose to produce sc-FOS.

2.1.2. Identity of the immobilising resin

The applicant's fructosyltransferase is produced as an immobilised enzyme preparation. Some information relevant to the resin is CCI therefore some details cannot be provided in this section.

Compared to enzymes in free solution, immobilised enzymes are more robust and resistant to environmental changes. Immobilised enzyme systems enable easy recovery of both enzymes and products, multiple reuses of enzymes, continuous operation of enzymatic processes, rapid termination of reactions, and a wider variety of reactor designs (Li *et al.* 2018).

FSANZ has reviewed data supplied by the applicant documenting the identity and characteristics of the immobilisation resin. Some information relevant to this resin are CCI, therefore some details cannot be provided in this section. The applicant confirmed that the resin used in the immobilised fructosyltransferase is suitable for food contact purposes and complies with the Council of Europe Policy Statement Concerning Ion Exchange and Adsorbant Resins in the Processing of Foodstuffs[2] and Regulation (EC) No 2023/20064[3] on good manufacturing practices for materials and articles intended to come into contact with foodstuffs. FSANZ assessment indicates that the resin is compliant with the Code.

2.2. Manufacturing process

2.2.1. Production of the enzyme

Enzymes produced from microorganisms are typically produced by controlled fermentation followed by removal of the production microorganism, purification, and concentration of the

[2] Policy statement concerning ion exchange and adsorbant resins in the processing of foodstuffs (Version 3 dated 28.01.2009) - European Directorate for the Quality of Medicines & HealthCare (edqm.eu)
[3] Regulation - 2023/2006 - EN - EUR-Lex (europa.eu)

enzyme. Final standardisation with stabilisers, preservatives, carriers, diluents, and other approved food-grade additives and ingredients is carried out after the purification and concentration steps. The formulated enzymes are referred to as enzyme preparations, which, depending upon the application in food, may be a liquid, semi-liquid, or dried product. Enzyme preparations may contain either one major active enzyme that catalyses one or more specific reactions during food processing or two or more active enzymes that catalyse different reactions (FAO/WHO 2020).

Information from the applicant demonstrated that immobilised fructosyltransferase is produced using a typical industrial process, in accordance with current Good Manufacturing Practice (cGMP) for Food[4] and the principles of Hazard Analysis and Critical Control Point (HACCP)[5]. All raw materials used in the fermentation and recovery processes are standard ingredients of food grade quality that meet predefined quality standards.

Details on the manufacturing process, raw materials and ingredients used in the production of the applicant's immobilised fructosyltransferase preparation were provided in the application, some as CCI.

2.2.2. Specifications for identity and purity

There are international general specifications for enzyme preparations used in the production of food. These have been established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in its Compendium of Food Additive Specifications (FAO JECFA Monographs 26 2021; FAO/WHO 2006) and in the Food Chemicals Codex (FCC 2022). Enzymes used as a processing aid need to meet these specifications. In addition, under JECFA, enzyme preparations must meet the specifications criteria contained in the individual monographs.

Schedule 3 of the Code also includes specifications for arsenic and heavy metals (section S3—4) if they are not already detailed within specifications in sections S3—2 or S3—3. The enzyme preparation does not exceed maximum levels for arsenic, cadmium, mercury (<1 mg/kg) and lead (<2 mg/kg) in section S3—4.

The applicant provided the results of analysis of three different batches of their immobilised fructosyltransferase. Table 2 provides a comparison of the results of those analyses with international specifications established by JECFA and Food Chemicals Codex, as well as those in the Code (as applicable). Based on those results, the enzyme preparation met all relevant specifications.

The fructosyltransferase is immobilised and any residual presence in in the final food is unlikely. Analytical data provided by the applicant showed no detectable protein, including any enzyme organic solids, in the final food (LOQ of 0.024%).

[4] known as cGMP, as distinct from GMP (which refers to the level of use of the enzyme).
[5] Compliant with Food Safety System Certification (FSSC) 22000 https://www.fssc.com/schemes/fssc-22000/

Table 2 Analysis of manufacturer's immobilised enzyme preparation compared to JECFA, Food Chemicals Codex, and Code specifications for enzymes.

Parameters	Tate and Lyle Batch Analyses	Specifications		
		JECFA[1]	FCC[2]	The Code[3]
Lead (mg/kg)	≤0.05	≤5	≤5	≤2
Arsenic (mg/kg)	≤1	-	-	≤1
Cadmium (mg/kg)	≤1	-	-	≤1
Mercury (mg/kg)	≤1	-	-	≤1
Coliforms (cfu/g)[4]	<10	≤30	≤30	-
Salmonella (in 25 g)	Negative	Absent	Negative	-
Escherichia coli (in 25 g)	Negative	Absent	-	-
Antimicrobial activity	Negative	Absent	-	-

[1]Joint FAO/WHO Expert Committee on Food Additives (FAO JECFA Monographs 26 (2021), [2]FCC Food Chemicals Codex (2022), [3]The code, Section S3—4, [4]cfu = colony forming units

2.3. Technological purpose

Immobilised fructosyltransferase is intended for use as a processing aid in the manufacture sc-FOS from sucrose. The applicant requested use of the enzyme preparation at GMP levels.

As identified by the IUBMB (see section 2.1), immobilised fructosyltransferase catalyses the fructosyl transfer from sucrose to produce sc-FOS (see BRENDA[6] enzyme database (Chang et al 2021) and Figure 1).

The technological purpose of immobilised fructosyltransferase stated by the applicant is catalysing the fructosyl transfer from sucrose to produce sc-FOS and this is consistent with the typical function of fructosyltransferases (Michel *et al.* 2015, Ibrahim 2021 DellaValle *et al.* 2018).

FOS have the following technological purposes:

- Sc-FOS are a mixture of oligosaccharides consisting of glucose linked to fructose units; links between fructose units are β-(1,2) (Hirayama *et al*. 1989). Sc-FOS are poorly digested in the human small intestine but are fermented in the colon by the resident microflora (Molis *et al.* 1996).
- Sc-FOS produced by fructosyltransferases are also used as low-calorie sweeteners and dietary fibres in various food products and enhance taste and texture (Michel *et al*. 2015, Ibrahim 2021 DellaValle *et al*. 2018).

The applicant provided sufficient CCI information on the physical and chemical properties of their enzyme preparation to demonstrate thermostability and pH stability when used for the purpose of producing sc-FOS.

2.4. Allergen considerations

Immobilised fructosyltransferase is not expected to pose a food allergenicity concern.

[6] EC explorer - BRENDA Enzyme Database (brenda-enzymes.org)

2.5. Food Technology Conclusion

The use of this immobilised fructosyltransferase as a processing aid for use in the production of sc-FOS from sucrose is consistent with its typical function of catalysing the fructosyl transfer from sucrose to produce sc-FOS.

Immobilised fructosyltransferase performs its technological purpose during the production of sc-FOS, after which it is not performing a technological function in the final food. It is therefore functioning as a processing aid for the purposes of the Code.

There are relevant identity and purity specifications for the enzyme in the Code, and the applicant provided evidence that the enzyme preparation meets these specifications.

3. Safety Assessment

The objectives of this safety assessment are to evaluate any potential public health and safety concerns that may arise from the use of the fructosyltransferase, associated with this *A. oryzae*, as a processing aid.

Some information relevant to this section is CCI, so full details cannot be provided in this public report.

3.1. History of use of the organism

A. oryzae is a filamentous fungus, isolated from soil and plants, which has been used extensively in both food and enzyme production. *A. oryzae* has been used for centuries in the fermentation of miso and soya sauce and the production of sake (Bourdichon et al. 2012, U.S EPA 1997). *A. oryzae* produces various native and recombinant enzymes that are widely used in food production and processing industries (Tanaka 2024) with the first patent for an enzyme derived from *A. oryzae* being published in 1911 (Kitagaki 2021).

While *A. oryzae* has been implicated in illness in severely ill and/or immunocompromised individuals, it is not considered to be pathogenic for healthy people (Barbesgaard et al 1992; Fitriana et al 2021), with JECFA and many countries which regulate the use of food enzymes, such as Australia, the USA, and Germany assessing the organism as safe for commercial use. FSANZ has approved the use of this species as an enzyme producer in previous applications including: A1246 (2022), A1241(2022), A1229 (2023), A606 (2008) and A561 (2006). No safety concerns were noted in these assessments. FSANZ notes that the specific strain used in this application is different to the strains assessed in previous applications. The production strain used in this application *is Aspergillus Oryzae* QHT-101 which was isolated from a food source.

Some experts consider *A. oryzae* to be a domesticated variant of *Aspergillus flavus*, a known human pathogen, which can produce aflatoxins. Misclassification between the species has occurred in the past due to their similar morphology and genome (Frisvad et al 2018). A common method for distinguishing the species is to test for the production of aflatoxins, as *A. oryzae* is not capable of producing them (Fitriana et al 2021; Han et al 2024). Aflatoxins were not detected in samples of *A. oryzae* when using a method with a limit of detection of 5 µg/kg. The applicant provided data that adequately demonstrates that the production strain is correctly identified as *A. oryzae.*

Mycotoxins present a health hazard to humans when ingested (Frisvad 2018). In certain conditions *A. oryzae* can produce mycotoxins (U.S EPA 1997). A mycotoxin analysis of three batches of the final product were used to show that no mycotoxins tested for were detected. Additionally, the production organism was not detected within the final enzyme preparation in three independent batches.

The microbiological assessment undertaken by FSANZ did not identify any public health and safety concerns associated with the use of *A. oryzae* as a source of fructosyltransferase.

3.2. Safety of the enzyme

3.2.1. History of safe use

There does not appear to be an established history of safe use for the specific fructosyltransferase that is the subject of this application, either free or when immobilised. However, closely related glucosyl hydrolase enzymes (invertase, beta-fructofuranosidases) have a long history of use in foods, including a beta-fructofuranosidase that is endogenously

produced by *Aspergillus fijiensis* and isolated for use as processing aids in food.

Multiple beta-fructofuranosidase enzymes from other microbial sources are currently permitted as processing aids in Schedule 18 of the Code.

3.2.2. Bioinformatic assessment of enzyme toxicity

A BLAST-P search was performed by the applicant using the fructosyltransferase protein sequence against sequences in the UniProt database[7] annotated as either toxin or venom (conducted Feb 2024). No sequences with sequence similarity above the nominated significance threshold (≥80 % sequence identity and ≥70 % coverage) were identified.

3.2.3. Evaluation of toxicity studies

No toxicity study reports were submitted to support the safety of the immobilised fructosyltransferase or free fructosyltransferase isolated from *A. oryzae*.

An *in-silico* digestion analysis was performed by the Applicant which indicates that the fructosyltransferase protein is likely to be extensively cleaved under gastric conditions. Furthermore, toxicity studies using closely related beta-fructofuranosidase isolated from *Aspergillus fijiensis* have previously been reviewed by FSANZ (A1212). No treatment-related adverse findings were reported.

Given the fructosyltransferase has no significant homology with known protein toxins, is likely to be extensively cleaved during digestion, is closely related glycosyl hydrolases previously reviewed by FSANZ without evidence of adverse effects, and there is limited potential for dietary exposure for the immobilised enzyme, the *A. oryzae* fructosyltransferase does not pose a toxicity risk to consumers under the proposed conditions of use.

There is no need to establish an ADI on the basis that there is only contact with the enzyme during food processing, and the likelihood of residues in the final food is negligible.

3.2.4. Potential for allergenicity

A FASTA search was performed using the amino acid sequence of fructosyltransferase using the AllergenOnline[8] database (queried Feb, 2024) using three sequence alignments: the full-length protein (more than 35% identity), an 80 mer sliding window (with an E-value[9] less than 1) and an 8 mer sliding window (exact match). Two allergen sequences were identified using these search parameters, both relating to the minor Sol I 2 food allergen from tomato (*Solanum lycopersicum*).

The beta-fructofuranosidase from tomato has been identified as a potential tomato food allergen based on IgE antibody reactivity (Foetisch et al., 2003). However, recombinant, non-glycosylated forms of beta-fructofuranosidase have been shown not to trigger immune responses in sera from patients with tomato allergy, indicating that host post-translational modification is an important feature in the allergenicity of tomato beta-fructofuranosidase (Westphal et al., 2003). The Applicant's fructosyltransferase is immobilised during FOS production and data has been provided to FSANZ to support that there is negligible carryover of fructosyltransferase enzyme into the final food.

Based on the available information, the enzyme preparation is not expected to pose a food

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[7] UniProt database: https://www.uniprot.org
[8] AllergenOnline: http://www.allergenonline.org/
[9] The E value (or Expect value) indicates the significance of a match found when searching a sequence database. The closer an E value gets to zero, the less likely an alignment could have been produced by chance.

allergenicity concern under the proposed conditions of use.

3.2.5. Immobilisation resin

FSANZ has reviewed data supplied by the applicant documenting the identity of the immobilisation resin, and considers there are no toxicity concerns associated with its use in the manufacture of immobilised fructosyltransferase. Some information relevant to this resin are CCI, therefore some details cannot be provided in this section.

3.4 Dietary Exposure

After evaluating the toxicity and allergenicity data for the fructosyltransferase, FSANZ considers immobilised fructosyltransferase does not represent a safety concern to consumers under the proposed conditions of use. The fructosyltransferase has limited potential for dietary exposure when used as immobilised fructosyltransferase for sc-FOS production As such, dietary exposure modelling was not needed for the safety assessment.

3.3. Safety Assessment Conclusions

The microbiological assessment undertaken by FSANZ did not identify any public health and safety concerns associated with the use of *A. oryzae* as a source of fructosyltransferase.

There is no need to establish an ADI on the basis that there is only contact with the enzyme during food processing, and the likelihood of residues in the final food is negligible.

The fructosyltransferase has limited potential for dietary exposure when used as immobilised fructosyltransferase for sc-FOS production and there are no toxicity concerns associated with the enzyme or the immobilisation resin. As such, dietary exposure modelling was not needed for the safety assessment.

The enzyme preparation is not expected to pose a food allergenicity concern under the proposed conditions of use.

Overall, FSANZ concludes there are no safety concerns from the use of immobilised fructosyltransferase derived from *A. oryzae* in the quantity and form consistent with its typical function in the manufacture of sc-FOS from sucrose.

4. References

Barbesgaard P, Heldt-Hansen HP, Diderichsen B (1992) On the safety of aspergillus oryzae: A review. Appl Microbiol Biotechnol 36(5):569-572

Bourdichon F, Casaregola S, Farrokh C, Frisvad JC, Gerds ML, Hammes WP, Harnett J, Huys G, Laulund S, Ouwehand A et al (2012) Food fermentations: Microorganisms with technological beneficial use. Int J Food Microbiol 154(3):87-97

DellaValle, D.M., Malek, A.M., Hunt, K.J., Peter, J.V.S., Greenberg, D., and Marriott, B.P., 2018. Low-calorie sweeteners in foods, beverages, and food and beverage additions: NHANES 2007–2012. Current developments in nutrition, 2(12), p.nzy024.

Enzyme nomenclature and classification: the state of the art - McDonald - 2023 - The FEBS Journal - Wiley Online Library

FAO/WHO (2020) Environmental Health Criteria 240. Principles and Methods for the Risk Assessment of Chemicals in Food. Chapter 9.1.4: Processing aids. Second Edition 2020. WHO, Geneva. Principles and methods for the risk assessment of chemicals in food (who.int)

Fitriana Y, Suharjo R, Swibawa IG, Semenguk B, Pasaribu LT, Hartaman M, Rwandini RA, Indriyati I, Purnomo P, Solikhin S (2021) Aspergillus oryzae and beauveria bassiana as entomopathogenic fungi of spodoptera litura fabricius (lepidoptera: Noctuidae) infesting corn in lampung, indonesia. Egypt J Biol Pest Co 31(1)

Foetisch K, Westphal S, Lauer I, Retzek M, Altmann F, Kolarich D, Scheurer S, and Vieths S. (2003). Biological activity of IgE specific for cross-reactive carbohydrate determinants. J Allergy Clin Immunol, 111(4), 889-896. https://doi.org/10.1067/mai.2003.173

Frisvad JC, Moller LLH, Larsen TO, Kumar R, Arnau J (2018) Safety of the fungal workhorses of industrial biotechnology: Update on the mycotoxin and secondary metabolite potential of *Aspergillus niger*, *Aspergillus oryzae*, and *Trichoderma reesei*. Appl Microbiol Biotechnol 102(22):9481-9515

Gupta, A., Sanwal, N., Bareen, M.A., Barua, S., Sharma, N., Olatunji, O.J., Nirmal, N.P. and Sahu, J.K., 2023. Trends in functional beverages: Functional ingredients, processing technologies, stability, health benefits, and consumer perspective. Food Research International, 170, p.113046.

Han DM, Baek JH, Choi DG, Jeon M-S, Eyun S-i, Jeon CO (2024) Comparative pangenome analysis of aspergillus flavus and aspergillus oryzae reveals their phylogenetic, genomic, and metabolic homogeneity. Food Microbiology 119

Hirayama M, Sumi N, Hidaka H. (1989) Purification and properties of fructo-oligosaccharides producing beta-fructofuranosidase from Aspergillus niger ATCC 20611. Agric Biol Chem. 1989;53:667–673.

Ibrahim, O.O., 2021. Technological aspects of fructo-oligosaccharides (FOS), production processes, physiological properties, applications, and health benefits. J Food Chem Nanotechnol.

Kitagaki H (2021) Medical application of substances derived from non-pathogenic fungi *Aspergillus oryzae* and a. Luchuensis-containing koji. J Fungi (Basel) 7(4)

Li, D., Chen, J., & Shi, Y. (2018). Advances on methods and easy separated support materials for enzymes immobilization. TrAC Trends in Analytical Chemistry. https://doi.org/10.1016/j.trac.2018.03.011

Mariela R. Michel, Rosa M. Rodríguez-Jasso, Cristóbal N. Aguilar, Silvia M. Gonzalez-Herrera, Adriana C. Flores- Gallegos and Raúl Rodríguez-Herrera (2015) Fructosyltransferase Sources, Production, and Applications for Prebiotics Production. In: Probiotics and Prebiotics in Human Nutrition and Health

Molis C, Flourie B, Ouarne F, Gailing MF, Lartigue S, Guibert A, Bornet F, Galmiche JP. Digestion, excretion, and energy value of fructooligosaccharides in healthy humans. Am J Clin Nutr. 1996;64:324–328.

Tadesse, S., 2012. Probiotics, prebiotics and synbiotics as functional food ingredients: production, health benefits and safety. Journal of Biologically Active Products from Nature, 2(3), pp.124-134.

Tanaka M (2024) Transcriptional and post-transcriptional regulation of genes encoding secretory proteins in aspergillus oryzae. Biosci Biotechnol Biochem 88(4):381-388

Trujillo Toledo, Luis & Martínez, Duniesky & Pérez Cruz, Enrique & Rivera-Intriago, Leonor & Nuñez, Jimmy & Pais-Chanfrau, José-Manuel. (2019). Fructosyltransferases and Invertases: Useful Enzymes in the Food and Feed Industries. 10.1016/B978-0-12-813280-7.00026-8.

U.S. EPA (U.S Environmental Protection Agency) (1997) Aspergillis oryzae final risk assessment: attachment I--Final risk assessment of Escherichia coli K-12 derivatives. Available online: https://www.epa.gov/sites/default/files/2015-09/documents/fra007.pdf

Westphal S, Kolarich D, Foetisch K, Lauer I, Altmann F, Conti A, Crespo JF, Rodriguez J, Enrique E, Vieths S, and Scheurer S. (2003). Molecular characterization and allergenic activity of Lyc e 2 (beta-fructofuranosidase), a glycosylated allergen of tomato. Eur J Biochem, 270(6), 1327-1337. https://doi.org/10.1046/j.1432-1033.2003.03503.x