# APPLICATION FOR THE AUTHORISATION OF D-PSICOSE 3-EPIMERASE FROM GENETICALLY MODIFIED *ESCHERICHIA COLI* STRAIN K-12 IN AUSTRALIA AND NEW ZEALAND

**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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# Application for the Authorisation of D-Psicose 3-Epimerase From Genetically Modified *Escherichia coli* Strain K-12 in Australia and New Zealand

## A. GENERAL REQUIREMENTS

In accordance with Section 3.1.1, General requirements, of the *Food Standards Australia New Zealand Application Handbook* (FSANZ, 2025), the following general information must be provided:

1. Format of the application;
2. Applicant details;
3. Purpose of the application;
4. Justification for the application;
5. Information to support the application;
6. Assessment procedure;
7. Confidential commercial information;
8. Other confidential information;
9. Exclusive capturable commercial benefit;
10. International and other national standards;
11. Statutory declaration; and
12. Checklist.

Each point is addressed in the following subsections.

## A.1 Format of the Application

### A.1.1 Information Related to Changes to Standard 1.3.3, Processing Aids

This application for an amendment to Standard 1.3.3, Processing Aids, and related schedules is prepared pursuant to Section 3.3.2, Processing aids, of the *Food Standards Australia New Zealand Application Handbook* (FSANZ, 2025), which requires the following structured format to assess an application for a new processing aid:

A. Technical information on the enzyme processing aid;

B. Information related to the safety of an enzyme processing aid;

C. Additional information related to the safety of an enzyme processing aid derived from a microorganism;

D. Additional information related to the safety of an enzyme processing aid derived from a genetically modified microorganism;

E. Information related to the dietary exposure to the processing aid.

The application is presented using the format outlined above. At the start of each section (Sections A to E), the information that must be addressed therein is specified in detail. Additionally, an executive summary for the application is provided as a separate electronic document to this application. The application has been prepared in English and has been submitted electronically as required by the *Food Standards Australia New Zealand Application Handbook* (FSANZ, 2025).

## A.2 Applicant Details

The applicant, Tate & Lyle Solutions USA LLC ("Tate & Lyle"), is a food ingredient manufacturer. The contact details for the company's representative are listed below.

Company name: Tate & Lyle Solutions USA LLC
Address: 5450 Prairie Stone Pkwy
Hoffman Estates, IL, 60192
USA

Telephone: [redacted]
Email: [redacted]

In addition, Intertek Health Sciences Inc. was involved in the preparation, submission, and stewardship of this application. The contact details for Intertek's representative are listed below.

Contact name: [redacted]
Address: Intertek Health Sciences Inc.
2233 Argentia Road, Suite 201
Mississauga, Ontario, Canada L5N 2X7

Telephone: [redacted]
Email: [redacted]

## A.3 Purpose of the Application

Tate & Lyle is submitting this application to Food Standards Australia New Zealand (FSANZ) to request an amendment to Standard 1.3.3, Processing Aids, of the *Food Standards Code* ("the Code") to include D-psicose 3-epimerase (EC 5.1.3.30) from a genetically modified (GM) strain of *Escherichia coli* strain K-12 as a processing aid for the production of D-allulose (also known as D-psicose). The recipient organism, *E. coli* strain K-12 W3310-TKO, is a GM derivative of *E. coli* strain K-12 W3110, one of the most widely used and well-characterised laboratory strains of *E. coli.* Strain K-12 W3110 is, in turn, derived from the wild-type *E. coli* strain K-12. The recipient organism was genetically modified to express a synthetic gene from *Desmospora* sp. 8437 encoding the D-psicose 3-epimerase enzyme. The gene was codon-optimised for expression by the *E. coli* production organism. The principal enzymatic activity of Tate & Lyle's enzyme is D-psicose 3-epimerase. Tate & Lyle requests that FSANZ expedite the review of this application.

## A.4 Justification for the Application

### A.4.1 Technological Function for the Processing Aid

D-psicose 3-epimerase catalyses the epimerisation of D-fructose to D-allulose. The enzyme reaction is metal-dependent and requires a divalent metal ion cofactor, [redacted] to exhibit activity. Thus, the technical effect of the D-psicose 3-epimerase enzyme is to produce D-allulose utilising D-fructose as a raw material.

### A.4.2 Costs and Benefits for Industry, Consumers, and Government Associated With Use of the Processing Aid

The enzymatic production of D-allulose *via* the catalytic activity of D-psicose 3-epimerase is an effective and economical method, allowing for large-scale production. D-allulose does not occur naturally in large amounts (Jürkenbeck *et al.*, 2022). Chemical synthesis of D-allulose is expensive and involves complex purification procedures and generates numerous toxic by-products. Unlike chemical synthesis, enzymatic production of D-allulose *via* C3 epimerisation of fructose has the advantage of lower costs, simplicity, and reduced environmental pollution (Xia *et al.,* 2021). These technological effects cannot be sufficiently met with the use of other enzymes or by other means; therefore, there exists a technological need for D-psicose 3-epimerase in food production. D-psicose 3-epimerase from *Microbacterium foliorum* is currently approved for use in Australia and New Zealand per Schedule 18 of the Code (FSANZ, 2026). Therefore, the permissibility of D-psicose 3-epimerase from a GM strain of *E. coli* (strain K-12) as a processing aid will provide food manufacturers and producers with alternative means of D-allulose production using a non-pathogenic and non-toxigenic source organism, which would promote healthy market competition and ultimately benefit the Australian/New Zealand consumer.

## A.5 Information to Support the Application

Information on the identity of D-psicose 3-epimerase from GM *E. coli* strain K-12, along with details on its manufacturing process, chemical and physical properties, and product specifications, is provided in Section B of the application. Information to support the safety of the processing aid is presented in Section C in accordance with the requirements listed in Section 3.3.2, Processing aids, of the *Food Standards Australia New Zealand Application Handbook* (FSANZ, 2025). The safety of D-psicose 3-epimerase was established based on the anticipated digestion of the enzyme to a significant degree and its lack of associated toxigenic or pathogenic concerns. The safety is further corroborated through lack of enzyme carryover to the final product and information related to potential allergenicity of the enzyme.

## A.6 Assessment Procedure

Tate & Lyle considers the General Procedure to be most appropriate route for assessing its application to include a GM strain, *E. coli* strain K-12, as a source organism for D-psicose 3-epimerase. Tate & Lyle anticipates that this application will involve amending Standard 1.3.3, Processing Aids, and Schedule 18 of the Code to include D-psicose 3-epimerase derived from GM *E. coli* strain K-12 as a processing aid.

## A.7 Confidential Commercial Information

Tate & Lyle requests that certain proprietary information be considered confidential commercial information (CCI). General summaries of the proprietary data are provided within this application and are summarised in Table A.7-1. Data requested to be treated as CCI have been removed and are provided within Appendix A **[CONFIDENTIAL]** in full.

All information presented in Appendix A **[CONFIDENTIAL]** is requested to remain confidential, as it holds significant commercial value to Tate & Lyle and includes proprietary details on the manufacture of the enzyme, the amino acid sequence, and enzyme activity.

**Table A.7-1 Information Requested to be Considered as Confidential**

| Section(s) | Description | Justification |
|---|---|---|
| 2 | Applicant and contact person contact details. | The contact details for the person responsible for the dossier are sensitive and should be treated as confidential. Public disclosure of this information is not required for the safety assessment of the processing aid. |
| Appendix A | Information on molecular mass, amino acid sequence, and optimum temperature and pH conditions.<br><br>Raw materials and processing aids used in the production process, detailed description of the production process, and process controls.<br><br>Data on compositional analysis.<br><br>Taxonomic identity of the production strain<br><br>Information on the methods used in the genetic modification of the source organism<br><br>Study demonstrating the absence of viable cells of the production strain and information on genetic stability. | The specific details of the described information and data are considered confidential and proprietary and are of significant commercial value to the applicant. Non-confidential summaries of the information are provided throughout the dossier. The publication of these data would provide a competitive advantage to other manufacturers. |
| Appendix A-1<br>Appendix A-2<br>Appendix A-3<br>Appendix A-4<br>Appendix A-5<br>Appendix A-6<br>Appendix A-7 | Enzyme Activity Methodology<br>Certificates of Analysis<br>Allergenicity of the Enzyme<br>Information About the Production Organism<br>Absence of Viable Cells of Production Strain<br>Absence of Residual DNA of Production Strain<br>Information on the Potential Toxicity of the Enzyme Processing Aid | These appendices contain sensitive information considered highly confidential and of significant commercial value to the applicant. Public disclosure of this information is not required for the safety assessment of the processing aid and the source organism. |

## A.8 Other Confidential Information

No other confidential information is contained within this application.

## A.9 Exclusive Capturable Commercial Benefit

Tate & Lyle anticipates that this application would confer exclusive capturable commercial benefit in accordance with Section 8 of the *Food Standards Australia New Zealand Act 1991*, as the production organism from which D-psicose 3-epimerase is derived (a GM strain of *E. coli* strain K-12) is a proprietary strain.

## A.10 International and Other National Standards

D-psicose 3-epimerase from *M. foliorum* is currently approved for use in Australia and New Zealand per Schedule 18 of the Code (FSANZ, 2026). Furthermore, D-psicose 3-epimerase from *Arthrobacter globiformis* was notified to the United States (U.S.) Food and Drug Administration (FDA) as Generally Recognized as Safe (GRAS) for use in the production of D-allulose and other keto sugars and received no questions from the agency (U.S. FDA, 2016). Tate & Lyle's D-psicose 3-epimerase derived from GM *E. coli* strain K-12, the subject of this application, has been evaluated by the European Food Safety Authority (EFSA) Panel on Food Enzymes (FEZ Panel) for use in the processing of fructose to produce D-allulose. Based on the absence of viable cells and residual DNA from the production organism in the enzyme, toxicological testing requirements were waived by the EFSA FEZ Panel. The Panel reviewed the available data on the enzyme and concluded that it does not give rise to safety concerns under the intended conditions of use (EFSA, 2025). Another D-psicose 3-epimerase from GM *E. coli* strain K-12 was evaluated by the EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP Panel) for use in the production of D-allulose. Similar to Tate & Lyle's enzyme, the enzyme is used in the immobilised form and residual amounts of enzyme total organic solids (TOS) are removed from the final D-allulose ingredient *via* purification. Upon review of the available data on the enzyme, the EFSA CEP Panel concluded that the enzyme does not give rise to safety concerns under the intended conditions of use (EFSA, 2021a).

## A.11 Statutory Declaration

Signed Statutory Declarations for Australia and New Zealand are provided in Appendix B.

## A.12 Checklist

A completed checklist relating to the information required for submission with this application based on the relevant guidelines in the *Food Standards Australia New Zealand Application Handbook* (FSANZ, 2025) are provided in Appendix C.

# B. TECHNICAL INFORMATION ON THE PROCESSING AID

In accordance with Part A of Section 3.3.2, Processing aids, of the *Food Standards Australia New Zealand Application Handbook* (FSANZ, 2025), the following technical information must be provided:

1. Information on the type of processing aid;
2. Information on the identity of the processing aid;
3. Information on the chemical and physical properties of the processing aid;
4. Manufacturing process;
5. Specification for identity and purity; and
6. Analytical method for detection.

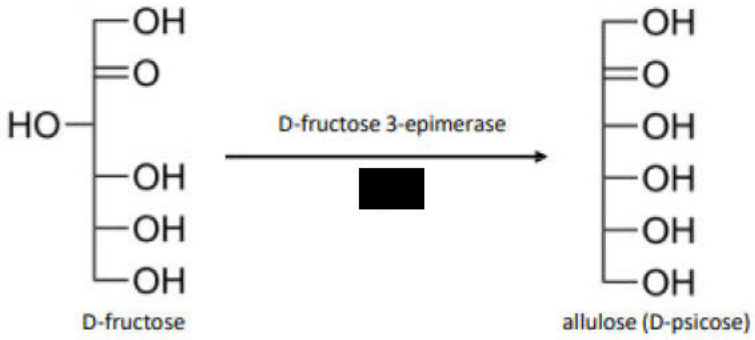
Each point is addressed in the following subsections.

## B.1 Information on the Type of Processing Aid

Tate & Lyle's D-psicose 3-epimerase is derived from GM *E. coli* strain K-12. D-psicose 3-epimerase is a metal-dependent enzyme that catalyses the reversible epimerisation of D-fructose at the C3 position to produce D-allulose (see Figure B.1-1).

Tate & Lyle's D-psicose 3-epimerase is manufactured as a lyophilised concentrate that is then immobilised onto food-grade resin. Based on the foregoing, Tate & Lyle's D-psicose 3-epimerase from GM *E. coli* strain K-12 expressing a synthetic, codon-optimised gene from *Desmospora* sp. 8437 would be categorised as an enzyme of microbial origin under Schedule 18 of the Code (FSANZ, 2026). The maximum intended use level of the enzyme preparation is 12.6 mg TOS/kg in D-fructose as a raw material (see Section F.1).

**Figure B.1-1 Enzymatic Reaction of D-Psicose 3-Epimerase Catalysing the Reversible Epimerisation of D-Fructose**




## B.2 Information on the Identity of the Processing Aid

Information on the identity of D-psicose 3-epimerase derived from the fermentation of a GM strain of *E. coli* (strain K-12), including the source organism, common and systematic name of the enzyme, synonyms, enzyme classification, and Chemical Abstracts Service registry numbers, is presented below in Table B.2-1. Tate & Lyle's D-psicose 3-epimerase has not been protein-engineered and is not modified by a post-translational process.

**Table B.2-1 Systemic Name and Registry Numbers of the Enzyme**

| | |
|---|---|
| **Source (Donor Organism):** | *Escherichia coli* strain K-12 expressing a synthetic, codon-optimised gene from *Desmospora* sp. 8437 |
| **Common/Accepted Name:** | D-psicose 3-epimerase |
| **Other Names:** | D-allulose 3-epimerase, fructose isomerase, D-fructose 3-epimerase, DPEase, DAEase |
| **Enzyme Classification Number of the Enzyme Commission (EC) of the International Union of Biochemistry and Molecular Biology (IUBMB):** | 5.1.3.30 |
| **Chemical/Systematic Name:** | D-psicose 3-epimerase |
| **Chemical Abstracts Service (CAS) Number:** | 1219591-85-1 |

## B.3 Information on the Chemical and Physical Properties of the Processing Aid

### B.3.1 Molecular Mass

D-psicose 3-epimerase from GM *E. coli* strain K-12 is manufactured as a lyophilised concentrate that is then immobilised onto food-grade resin. The protein pattern of the liquid concentrate of the D-psicose 3-epimerase enzyme prior to immobilisation was evaluated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE). Results of the SDS-PAGE analysis and the approximate molecular weight of the D-psicose 3-epimerase enzyme are provided in Appendix A **[CONFIDENTIAL]**.

### B.3.2 Amino Acid Sequence

Details on the determination of the gene encoding the D-psicose 3-epimerase enzyme and the full amino acid sequence of the enzyme are provided in Appendix A **[CONFIDENTIAL]**. The synthetic gene encoding the D-psicose 3-epimerase enzyme was designed based on a native gene from *Desmospora* sp. 8437. The gene was codon-optimised for expression by the *E. coli* production organism. A sequence homology search was conducted with the amino acid sequence of Tate & Lyle's D-psicose 3-epimerase using the Basic Local Alignment Search Tool (BLAST) hosted by the National Center for Biotechnology Information (NCBI) against the non-redundant protein database. The amino acid sequence of the enzyme was demonstrated to share significant sequence homology (96.19% identity and 100% query coverage) with the native D-tagatose 3-epimerase from *Desmospora* sp. 8437 on which it is based.

### B.3.3 Properties of the Enzyme

#### *B.3.3.1 Information on the Principal Enzymatic Activity*

D-psicose 3-epimerase catalyses the reversible epimerisation of D-fructose at the C3 position to produce D-allulose (see Figure B.1-1). The enzyme acts by tautomerising the proton at the C3 position of D-fructose, resulting in an enol intermediate. The intermediate is then selectively re-protonated at C3, forming D-allulose. D-psicose 3-epimerase is a metal-dependent enzyme that requires a divalent metal ion cofactor to exhibit activity. Several metal ions can act as cofactors, [redacted] is used as the metal ion cofactor in the production of allulose due to its safety profile. D-psicose 3-epimerase does not have any other subsidiary or side activities.

D-psicose 3-epimerase is specifically characterised by its D-psicose 3-epimerase enzyme activity. Tate & Lyle has established a specification limit for D-psicose 3-epimerase activity, and analytical data supporting the enzyme activity levels are provided in Section B.5.2.1. D-psicose 3-epimerase activity is measured using the assay method described in Appendix A-1 **[CONFIDENTIAL]**. D-psicose 3-epimerase activity is reported on an enzyme unit per gram basis. One D-psicose 3-epimerase unit is defined as the amount of the enzyme that will convert 1 µmole of D-fructose to D-allulose per minute under the conditions of the assay.

#### *B.3.3.2 Activity of the Enzyme Under the Conditions of Intended Use*

The optimal pH and temperature conditions for D-psicose 3-epimerase produced by GM *E. coli* strain K-12 were determined experimentally (see Appendix A **[CONFIDENTIAL]**).

#### *B.3.3.3 Subsidiary and/or Side Activities*

The principal activity of D-psicose 3-epimerase is to catalyse the reversible epimerisation of D-fructose at the C3 position to produce D-allulose. D-psicose 3-epimerase does not contain any other subsidiary or side activities.

#### *B.3.3.4 Fate of the Enzyme During Food Processing and Its Behaviour in the Food Matrix*

D-psicose 3-epimerase catalyses the reversible epimerisation of D-fructose at the C3 position to produce D-allulose. Tate & Lyle's D-psicose 3-epimerase is intended for use in the immobilised form in the processing of fructose to produce D-allulose. The enzyme is electrostatically bound onto a resin such that it can be utilised continuously to produce D-allulose in a column-based operation. Transfer of the immobilised enzyme into the crude D-allulose syrup is expected to be negligible. Furthermore, once the epimerisation process is complete, the crude D-allulose syrup undergoes separation, purification, and refinement. These processes eliminate residual proteins and particles of the immobilisation resin present in the syrup. Therefore, the likelihood of residual enzyme persisting in the final food ingredient is low. Three batches of D-allulose syrup produced with Tate & Lyle's D-psicose 3-epimerase were analysed and demonstrated to be absent of residual protein (less than the limit of detection [LOD] of 3.4 mg/kg) (see Appendix A-2).

## B.4 Manufacturing Process

### B.4.1 Manufacturing Process of the Processing Aid

#### *B.4.1.1 Identity of Raw Materials and Processing Aids*

A list of raw materials, processing aids, and purification aids used in the production of D-psicose 3-epimerase is provided in Appendix A **[CONFIDENTIAL]**. All processing aids are high quality and acceptable for use in the manufacture of enzymes intended for use as processing aids.

#### *B.4.1.2 Description of the Key Steps Involved in the Production Process*

D-psicose 3-epimerase is manufactured in accordance with current Good Manufacturing Practice, and the process is certified to FSSC 22000. The enzyme is produced using food-grade materials and quality-controlled fermentation and purification/recovery processes. The production strain has been deposited in a recognised culture collection.

The production of the immobilised D-psicose 3-epimerase follows 3 steps: culture of the production strain, downstream processing, and immobilisation of the enzyme. An overview of the manufacturing process is presented in Appendix A **[CONFIDENTIAL]**, and a brief description of the 3 key steps is provided below.

<u>Culture of the Production Strain</u>

In the first step, the production strain is fermented in the cultured medium under the appropriate fermentation conditions. The culture is then used as inoculum for seed fermentation. Seed fermentation is conducted under controlled conditions until sufficient biomass has been obtained. In the subsequent main fermentation step, the production strain from the seed culture step is inoculated in the same fermentation medium. Main fermentation is operated under submerged fed-batch conditions.

Downstream Processing

At the end of fermentation, the fermentation broth is centrifuged. The cells are collected and mechanically pulverised then centrifuged to obtain a supernatant containing the D-psicose 3-epimerase enzyme. The supernatant is then filtered for removal of residual DNA from the production organism after which it is concentrated by ultra-filtration and freeze-dried to stabilise the enzyme.

Immobilisation of the Enzyme

To obtain the immobilised enzyme, a food-grade ion-exchange resin is conditioned with the appropriate substances and added to the supernatant containing the enzyme for adsorption.[1] Following enzyme adsorption, the resin is then washed, packaged, and utilised for the epimerization of D-fructose to D-allulose.

#### *B.4.1.3 Process Controls and Quality Assurance Procedures*

A Hazard Analysis and Critical Control Point (HACCP) plan is in place for the manufacture of D-psicose 3-epimerase produced with GM *E. coli* strain K-12. The critical control points (CCPs) have been identified, and measures have been put in place to prevent the identified hazards. The HACCP plan consists of series of processes that are identified as CCPs to ensure adherence with the established manufacturing process and produce a high-quality, consistent product. These include measures to ensure that potential residual amounts of the production strain are not transferred to the enzyme. Furthermore, each manufactured batch of the enzyme is analysed for conformance to the specifications set out in Section B.5.1. Batches that do not meet the specifications are not released for further processing. The quality control steps are presented in Appendix A [CONFIDENTIAL].

## B.5 Specification for Identity and Purity

### B.5.1 Product Specifications

The specifications for D-psicose 3-epimerase from GM *E. coli* strain K-12 are provided in Table B.5.1-1. The specifications comply with the purity requirements for enzyme preparations established by FCC (2026) and JECFA (2006a). All methods of analysis are internationally recognised or validated methods. The enzyme activity for D-psicose 3-epimerase is determined by the amount of enzyme required to convert D-fructose to D-allulose (see Section B.3.3). The assay method is provided in Appendix A-1 [CONFIDENTIAL].

**Table B.5.1-1 Specifications for D-Psicose 3-Epimerase From Genetically Modified *Escherichia coli* Strain K-12**

| Specification Parameter | Specification | Method of Analysis |
|---|---|---|
| Appearance | White-to-coloured powder; may contain larger agglomerates | Visual inspection |
| *Escherichia coli*[1] | Negative in 25 g | USP 38–NF 33 |
| Total coliforms[2] | ≤30 CFU/g | FDA BAM Chapter 4: Enumeration of Escherichia coli and the Coliform Bacteria |
| *Salmonella* sp.[3] | Negative in 25 g | BioMerieux VIDAS® UP *Salmonella* (SPT) assay method |
| Antimicrobial activity | Negative | JECFA (2006b) |

[1] No cross-linking agents are used during the immobilisation process to the ion-exchange resin.

**Table B.5.1-1 Specifications for D-Psicose 3-Epimerase From Genetically Modified *Escherichia coli* Strain K-12**

| Specification Parameter | Specification | Method of Analysis |
|---|---|---|
| Lead[4] | <1 mg/kg | AOAC 2011.19, 993.14, and 2015.01 (Modified) |
| Arsenic[4] | <1 mg/kg | AOAC 2011.19, 993.14, and 2015.01 (Modified) |
| Mercury[4] | <0.5 mg/kg | AOAC 2011.19, 993.14, and 2015.01 (Modified) |
| Cadmium[4] | <0.5 mg/kg | AOAC 2011.19, 993.14, and 2015.01 (Modified) |

AOAC = Association of Official Analytical Chemists; CFU = colony-forming units; FCC = *Food Chemicals Codex;* FDA BAM = *Food and Drug Administration Bacteriological Manual*; ICP-MS = Inductively Coupled Plasma Mass Spectrometry; JECFA = Joint FAO/WHO Expert Committee on Food Additives; LOD = limit of detection; LOQ = limit of quantitation; NA = not applicable; NF = *National Formulary*; USP = *United States Pharmacopeia*.

[1] LOD = NA; LOQ = NA.

[2] LOD = 10 CFU/g or mL; LOQ = 25 to 250 CFU/g or mL.

[3] LOD = 0.2 to 2 CFU/25 g; LOQ = NA.

[4] LOQ for the determination of heavy metals by ICP-MS method is 5 µg/kg for Pb, Hg, Cd and 10 µg/kg for As.

## B.5.2 Product Analysis

### *B.5.2.1 Batch Analyses*

D-psicose 3-epimerase is manufactured as a lyophilised concentrate that is immobilised onto food-grade resin. Analytical data on 3 non-consecutive production-scale batches of immobilised D-psicose 3-epimerase are presented in Table B.5.2.1-1 and demonstrate that the manufacturing process produces a consistent product that meets the product specifications defined in Section B.5.1. All heavy metals (*i.e.,* lead, arsenic, mercury, and cadmium) and microbiological contaminants were below the LOD and/or in compliance with the specifications for the enzyme. In addition, all batches were negative for antimicrobial activity. The certificates of analysis are provided in Appendix A-2 **[CONFIDENTIAL]**. All methods of analysis are internationally recognised or validated methods.

**Table B.5.2.1-1 Batch Analyses for 3 Non-Consecutive Production-Scale Batches of D-Psicose 3-Epimerase From Genetically Modified *Escherichia coli* Strain K-12**

| Specification Parameter | Specification Limit | The Code (Section S3-4) | Manufacturing Lot | | | Method of Analysis |
|---|---|---|---|---|---|---|
| | | | CDX-088#02 | TL-CDX-088#01 | TL-CDX-088#02 | |
| **Physical Properties** | | | | | | |
| Appearance | White-to-coloured powder; may contain larger agglomerates | - | Tan free-flowing powder | | | Visual inspection |
| **Microbiological Specifications** | | | | | | |
| *Escherichia coli*[1] | Negative in 25 g | - | Negative | Negative | Negative | USP 38–NF 33 |
| Total coliforms[2] | ≤30 CFU/g | - | 10 | <10 | <10 | FDA BAM Chapter 4: Enumeration of Escherichia coli and the Coliform Bacteria |
| *Salmonella* sp.[3] | Negative in 25 g | - | Negative | Negative | Negative | BioMerieux VIDAS® UP Salmonella (SPT) assay method |
| Antimicrobial activity | Negative | - | Negative | Negative | Negative | JECFA (2006b) |
| **Heavy Metals** | | | | | | |
| Lead[4] | <1 mg/kg | ≤2 | Compliant with Code | | | AOAC 2011.19, 993.14, and 2015.01 (Modified) |
| Arsenic[4] | <1 mg/kg | ≤1 | Compliant with Code | | | AOAC 2011.19, 993.14, and 2015.01 (Modified) |
| Mercury[4] | <0.5 mg/kg | ≤1 | Compliant with Code | | | AOAC 2011.19, 993.14, and 2015.01 (Modified) |
| Cadmium[4] | <0.5 mg/kg | ≤1 | Compliant with Code | | | AOAC 2011.19, 993.14, and 2015.01 (Modified) |

AOAC = Association of Official Analytical Chemists; CFU = colony-forming units; FDA BAM = *Food and Drug Administration Bacteriological Manual*; ICP-MS = Inductively Coupled Plasma Mass Spectrometry; LOD = limit of detection; LOQ = limit of quantitation; NA = not applicable; NF = *National Formulary*; USP = *United States Pharmacopeia*.

[1] LOD = NA; LOQ = NA.

[2] LOD = 10 CFU/g or mL; LOQ = 25 to 250 CFU/g or mL.

[3] LOD = 0.2 to 2 CFU/25 g; LOQ = NA.

[4] LOQ for the determination of heavy metals by ICP-MS method is 5 µg/kg for Pb, Hg, Cd and 10 µg/kg for As

Additional compositional data for the same 3 non-consecutive production-scale batches of D-psicose 3-epimerase are provided in Appendix A **[CONFIDENTIAL]**. The enzyme was confirmed to comprise ash, protein, and water.

Additional analyses were conducted to demonstrate the absence of residual impurities originating from the manufacturing process in the immobilised D-psicose 3-epimerase. Analytical data from the same 3 non-consecutive production-scale batches of D-psicose 3-epimerase are provided in Appendix A **[CONFIDENTIAL]**. All processing impurities were below the limit of quantitation and were therefore concluded to be absent in the final enzyme. The certificates of analysis are provided in Appendix A-2 [**CONFIDENTIAL]**.

## B.6 Analytical Method for Detection

According to Section 3.3.2, Processing aids, of the *Food Standards Australia New Zealand Application Handbook* (FSANZ, 2025), an analytical method for detection is not required in the case of an enzymatic processing aid, and this section is therefore not relevant to D-psicose 3-epimerase derived from GM *E. coli* strain K-12.

# C. INFORMATION RELATED TO THE SAFETY OF AN ENZYME PROCESSING AID

In accordance with Part C of Section 3.3.2, Processing aids, of the *Food Standards Australia New Zealand Application Handbook* (FSANZ, 2025), the following safety information must be provided for enzyme processing aids:

1. General information on the use of the enzyme as a food processing aid in other countries;
2. Information on the potential toxicity of the enzyme processing aid;
3. Information on the potential allergenicity of the enzyme processing aid;
4. Safety assessment reports prepared by international agencies or other national government agencies, if applicable;

Each point is addressed in the following subsections.

## C.1 General Information on the Use of the Enzyme as a Food Processing Aid in Other Countries

D-psicose 3-epimerase from *M. foliorum* is currently approved for use in Australia and New Zealand per Schedule 18 of the Code (FSANZ, 2026). D-psicose 3-epimerase from *A. globiformis* was notified to the U.S. FDA as GRAS for use in the production of D-allulose and other keto sugars and received no questions from the agency (U.S. FDA, 2016). Tate & Lyle's D-psicose 3-epimerase derived from the fermentation of GM *E. coli* strain K-12 was evaluated by the EFSA FEZ Panel for use in the processing of fructose for the production of allulose. Based on the absence of viable cells and DNA from the production organism in the enzyme, toxicological testing requirements were not considered necessary. The EFSA FEZ Panel reviewed the available data on the enzyme and concluded that it does not give rise to safety concerns under the intended conditions of use (EFSA, 2025). Another D-psicose 3-epimerase from GM *E. coli* strain K-12 was evaluated by the EFSA CEP Panel for use in the production of D-allulose. Similar to Tate & Lyle's enzyme, the enzyme is used in the immobilised form and residual amounts of enzyme TOS are removed from the final D-allulose ingredient *via* purification. Upon review of the available data on the enzyme, the EFSA CEP Panel concluded that the enzyme does not give rise to safety concerns under the intended conditions of use (EFSA, 2021a).

## C.2 Information on the Potential Toxicity of the Enzyme Processing Aid

The potential toxicity of D-psicose 3-epimerase was addressed according to Section 3.3.2, Processing aids, of the *Food Standards Australia New Zealand Application Handbook* (FSANZ, 2025), which requires information on the enzyme's prior history of human consumption and its similarity to proteins with a history of safe human consumption, information on the similarity of the amino acid sequence to known protein toxins and allergens, and information on the stability of the enzyme in digestion models.

A sequence homology was conducted using the amino acid sequence of D-psicose 3-epimerase using the BLAST Tool hosted by the NCBI to identify similar proteins with history of safe consumption by humans. The enzyme was found to share significant sequence homology (100% query coverage and 96.19% identity) with the native protein from *Desmospora* sp. 8437, from which the synthetic gene encoding Tate & Lyle's enzyme was derived, as well as other D-psicose 3-epimerases from other bacterial species such as *Kroppenstedtia eburnean, Novibacillus thermophilus, Siminovitchia* sp. The sequence homology search is provided in Appendix A-7. Although another D-psicose 3-epimerase from *M. foliorum* is permitted for use in the production of D-allulose in Australia and New Zealand, the enzyme is used in its immobilised form for conversion of D-fructose to D-allulose. As such, there is negligible likelihood of consumer exposure to enzyme TOS. As such, it was concluded that Tate & Lyle's D-psicose 3-epimerase does not share similarity with proteins with a history of safe consumption by humans.

A toxin sequence alignment query of the amino acid sequence of D-psicose 3-epimerase from GM *E. coli* strain K-12 was conducted using BLAST. The sequence was searched against downloaded protein sequences obtained from a curated database of 8,572 venom proteins and toxins[2] maintained by UniProt (UniProt Consortium, 2025, 2026). Sequence alignments were considered significant using the criteria reported by EFSA (2024) for identifying genes of concern (*i.e.,* ≥80% identity and ≥70% coverage). The sequence homology searches did not identify any significant similarity, indicating that the D-psicose 3-epimerase enzyme from GM *E. coli* strain K-12 does not contain any significant sequence homology to any known animal protein or toxin. The sequence homology search reports are provided in Appendix A-7 **[CONFIDENTIAL].**

[2] UniProtKB/Swiss-Prot; available at: https://www.uniprot.org/ using search terms: ((cc_tissue_specificity:venom) OR (keyword:KW-0800)) AND (reviewed:true).

As Tate & Lyle's D-psicose 3-epimerase does not have a history of safe human consumption nor share similarities with other proteins with a history of safe human consumption, the stability of the enzyme to degradation in appropriate gastric digestion systems was investigated *in silico.* The potential digestion of D-psicose 3-epimerase from GM *E. coli* strain K-12 was predicted using Expasy's PeptideCutter.[3] The potential cleavage sites in the amino acid sequence of the enzyme were analysed using pepsin at pH 1.3 and >2 to simulate gastric conditions and using trypsin to simulate intestinal conditions. The cleavages estimated in the 525-residue sequence using pepsin at pH 1.3, pH >2, and for trypsin were 54, 80, and 36, respectively (see Appendix A-7 **[CONFIDENTIAL]**). Thus, it can be predicted that any residual D-psicose 3-epimerase enzyme consumed would be extensively digested in the stomach and further degraded in the small intestine.

Furthermore, Tate & Lyle has demonstrated that there is negligible likelihood of consumer exposure to enzyme TOS, viable cells of the production organism, residual DNA from the production organism, or residues from the immobilisation system. As demonstrated in Section B.3.3.4, the presence of enzyme TOS was below the LOD of 3.4 mg/kg in multiple batches of the final product (D-allulose). Therefore, considering that the enzyme is intended for use only in the immobilised form, any residual carryover of the enzyme from the immobilised resin into the final product (D-allulose) is expected to be negligible.

No significant sequence homology was identified between the enzyme and known protein and allergens (see Section C.3). Furthermore, it was predicted that any residual D-psicose 3-epimerase enzyme consumed would be extensively digested in the stomach and further degraded in the small intestine. Moreover, Tate & Lyle has demonstrated the absence of enzyme TOS and viable cells of and residual DNA from the production organism in the final product (D-allulose). As such, toxicological studies were not considered necessary for the enzyme. Similarly, in the assessment of D-psicose 3-epimerase from *M. foliorum* by FSANZ, it was noted that the applicant provided evidence that there is negligible likelihood of consumer exposure to the production organism, the intact enzyme, or residues from the immobilized cell system. No significant homology was found with any known toxins or allergens. As such, FSANZ concluded that toxicological studies of D-psicose 3-epimerase were not necessary. Furthermore, the safety of Tate & Lyle's D-psicose 3-epimerase for use in the production of D-allulose has also been evaluated by the EFSA FEZ Panel (EFSA, 2025). The enzyme is used in an immobilised form, and residual amounts of enzyme TOS are removed during the production of D-allulose. The EFSA FEZ Panel recognised that dietary exposure to the enzyme is not anticipated, and standard toxicological studies on the enzyme were not deemed necessary. Based on the data provided, the EFSA FEZ Panel concluded that the enzyme does not give rise to safety concerns under the intended conditions of use.

## C.3 Information on the Potential Allergenicity of the Enzyme Processing Aid

The potential allergenicity of D-psicose 3-epimerase from GM *E. coli* strain K-12 was assessed by investigating sequence homology between the enzyme and known food allergens using the methodology described by FAO/WHO (2001), Codex Alimentarius (2003, 2009), and EFSA (2017).

To investigate the possibility of allergenic cross-reactivity of D-psicose 3-epimerase from GM *E. coli* strain K-12, an *in silico* assessment of sequence homology of the amino acid sequence of the enzyme against known allergens was conducted using the methodology described by FAO/WHO (2001), Codex Alimentarius (2003, 2009), and EFSA (2017). In accordance with these guidelines, the following sequence homology searches were conducted against the AllergenOnline database (Version 23)[4]:

[3] https://web.expasy.org/peptide_cutter/.
[4] http://www.allergenonline.org.

1. A “sliding window” of 80–amino acid sequences (segments 1–80, 2–81, 3–82, *etc*.) derived from each full-length amino acid sequence of the enzyme (search parameters: minimum identity cut-off = 35%);

2. An 8–amino acid exact match; and

3. The full-length amino acid sequence (search parameters: FASTA36; E-value cut-off = 1 and maximum alignment of 20).

The searches were conducted on 26 March 2026. From the sliding window searches of 80 amino acids with D-psicose 3-epimerase, no matches were identified that shared ≥35% identity over 80 amino acids with a known allergenic protein from AllergenOnline. No 8–amino acid exact matches were identified. Furthermore, no matches were identified that shared ≥50% identity over the full-length amino acid sequence. The raw outputs of the allergenicity searches are provided in Appendix A-3 **[CONFIDENTIAL]**.

The allergenicity of D-psicose 3-epimerase was further considered through a search of available scientific literature. Search terms included synonyms for allergenicity (allerg* or hypersensitiv* OR hypersensitiz* OR hypersensitis* or "*crossreacti*" or "*cross-reacti*") and common names characterising the enzyme (*e.g.*, D-psicose 3-epimerase or D-allulose 3-epimerase). The search was conducted on 26 March 2026 using the following databases: AdisInsight: Trials, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, National Technical Information Service, ToxFile®, and Toxicology Abstracts. The complete literature search report is provided in Appendix A-3 **[CONFIDENTIAL]**.

Upon review of the literature search results, no information was identified on oral or respiratory sensitisation or elicitation reactions of D-psicose 3-epimerase from GM *E. coli* strain K-12.

Based on the information, no evidence was identified that would suggest D-psicose 3-epimerase from GM *E. coli* strain K-12 would be cross-reactive with known allergens. Although the risk of allergic reactions by dietary exposure to this enzyme cannot be excluded, the risk is considered low based on the intended conditions of its use in food processing. Furthermore, it should be noted that the enzyme will only be used in the immobilised form, and as described in Section C, analytical data have confirmed negligible carryover of enzyme TOS into the final product, further limiting potential exposure to the enzyme. There is no evidence from the available scientific literature to indicate any allergenicity to D-psicose 3-epimerase in consumers. Furthermore, it is expected that the enzyme would be denatured if carried over into the final D-allulose product under the conditions of food processing and therefore would not have the potential to result in allergenicity in consumers of the final foods. Based on this information, no evidence exists to indicate that D-psicose 3-epimerase from GM *E. coli* strain K-12 would produce an allergenic response following its use in food processing.

## C.4 Safety Assessment Reports Prepared by International Agencies or Other National Government Agencies

D-psicose 3-epimerase from *M. foliorum* is currently approved for use in Australia and New Zealand per Schedule 18 of the Code (FSANZ, 2026). Furthermore, D-psicose 3-epimerase from *Arthrobacter globiformis* was notified to the United States (U.S.) Food and Drug Administration (FDA) as Generally Recognized as Safe (GRAS) for use in the production of D-allulose and other keto sugars and received no questions from the agency (U.S. FDA, 2016). Tate & Lyle’s D-psicose 3-epimerase derived from GM *E. coli* strain K-12, the subject of this application, has been evaluated by the European Food Safety Authority (EFSA) Panel on Food Enzymes (FEZ Panel) for use in the processing of fructose to produce D-allulose. Based on the absence of viable cells and residual DNA from the production organism in the enzyme, toxicological testing requirements were waived by the EFSA FEZ

Panel. The Panel reviewed the available data on the enzyme and concluded that it does not give rise to safety concerns under the intended conditions of use (EFSA, 2025). Another D-psicose 3-epimerase from GM *E. coli* strain K-12 was evaluated by the EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP Panel) for use in the production of D-allulose. Similar to Tate & Lyle's enzyme, the enzyme is used in the immobilised form and residual amounts of enzyme total organic solids (TOS) are removed from the final D-allulose ingredient *via* purification. Upon review of the available data on the enzyme, the EFSA CEP Panel concluded that the enzyme does not give rise to safety concerns under the intended conditions of use (EFSA, 2021a).

# D. INFORMATION RELATED TO THE SAFETY OF AN ENZYME PROCESSING AID DERIVED FROM A MICROORGANISM

In accordance with Part D of Section 3.3.2, Processing aids, of the *Food Standards Australia New Zealand Application Handbook* (FSANZ, 2025), the following safety information must be provided for enzyme processing aids:

1. Information on the source microorganism;
2. Information on the potential pathogenicity and toxicity of the source microorganism; and
3. Information on the genetic stability of the source organism.

Each point is addressed in the following subsections.

## D.1 Information on the Source Microorganism

D-psicose 3-epimerase is obtained from GM *E. coli* strain K-12. The host organism is derived from a strain of *E. coli* that has undergone gene deletion to confer resistance to phage contamination. No other genetic modifications have been made to the host organism. *E. coli* strain K-12 is widely recognised as a non-pathogenic and non-toxigenic organism belonging to Risk Group 1 under the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (NIH, 2024). Furthermore, *E. coli* strain K-12 and its derivatives meet the safety requirements of Good Industrial Large-Scale Practice of the Organisation for Economic Co-operation and Development (OECD, 1992).

### D.1.1 Taxonomic Identity of the Production Strain

The taxonomic identity of the production organism was determined *via* whole genome sequence (WGS) analysis. The strain was cultured on Lysogeny Broth agar. Subsequently, DNA was extracted for construction of the WGS. After assembly of the WGS, the average nucleotide identity of the strain was compared to the reference genome of *E. coli* strain K-12. The details of the WGS methodologies and findings are provided in Appendix A **[CONFIDENTIAL]**. The results demonstrate that the production strain is taxonomically classified as *E. coli*. The certificate of deposit is provided in Appendix A-4 **[CONFIDENTIAL]**. The taxonomic classification of the source organism is presented in Figure D.1-1.

**Figure D.1-1 Taxonomy of Source Organism**

Kingdom: Bacteria
Phylum: Pseudomonadota
Class: Gammaproteobacteria
Order: Enterobacterales
Family: Enterobacteriaceae
Genus: *Escherichia*
Species: *Escherichia coli*
Strain: K-12

### D.1.2 Details of Documented History of Use With Absence of Human Health Adverse Effects

The strain from which the recipient organism is derived is a non-pathogenic and non-toxigenic member of the well-characterised family Enterobacteriaceae and has a long history of use for the fermentation of protein and small-molecule products for use in food and beverage products globally. *E. coli* strain K-12 is a base strain that has been determined to be safe for biotechnology and food industry laboratory use; others base strains determined to be safe include strains B (BL21), C, and W. These 4 strains and their derivatives have been designated as Biosafety Level 1 or Risk Group 1 organisms according to biological safety guidelines (Jeong *et al.,* 2009; Archer *et al.,* 2011), as they are well-characterised, do not cause disease in healthy adults (NIH, 2024), and do not colonise the human gut (Bauer *et al.,* 2008). The Joint FAO/WHO Expert Committee on Food Additives has also indicated that *E. coli* is an acceptable microorganism to produce enzymes used as processing aids in food production (JECFA, 2020, 2021).

## D.2 Information on the Pathogenicity and Toxicity of the Source Microorganism

According to the guidelines developed by Pariza and Foster (1983), Pariza and Johnson (2001), and IFBC (1990), the primary consideration in evaluating the safety of an enzyme preparation derived from a microbial source is the safety of the production strain. The safety of a production strain is addressed primarily by evaluating its toxigenic potential, pathogenicity, and antibiotic production. According to the

established guidelines, the safety of the enzyme preparation itself also should be assessed in part *via* analytical testing to ensure absence of toxic constituents and antibiotic activity. The potential toxigenicity and pathogenicity of *E. coli* strain K-12 and the impact on the manufactured D-psicose 3-epimerase enzyme are discussed below.

### D.2.1 Production Strain Pathogenicity and Toxigenicity

The potential for derivatives of *E. coli* strain K-12 to present a hazard to human health has been extensively investigated and is the subject of several reviews by various scientific and regulatory agencies. The U.S. Environmental Protection Agency (EPA) has conducted an extensive risk assessment on *E. coli* strain K-12 derivatives in support of the *Microbial Products of Biotechnology Final Regulation Under the Toxic Substances Control Act* (U.S. EPA, 1997a). The focus of the EPA's risk assessment (U.S. EPA, 1997a) was on occupational exposure and non–food-related uses (*e.g.*, the production of specialty and commodity chemicals). However, the overall conclusions of the risk assessment are applicable to the discussion of the toxigenic and pathogenic potential of *E. coli* strain K-12 derivatives when used in the production of enzymes intended for use as processing aids in food production.

The U.S. EPA reviewed several studies from the available scientific literature demonstrating that *E. coli* strain K-12 is not able to colonise the human intestinal tract under normal conditions (Anderson, 1975; Smith, 1975; Levy *et al.,* 1980). *E. coli* strain K-12 does not have the cell wall components necessary to adhere to the mucosal surface of the colon and is therefore out-competed by indigenous intestinal microorganisms. To cause disease, a microorganism must be able to colonise a site of the human body and express virulence factors. On the basis of intestinal colonisation alone, *E. coli* strain K-12 has a low likelihood of acting pathogenic to humans or other mammals. *E. coli* strain K-12 also does not produce toxins that affect humans (U.S. EPA, 1997b). Upon review of the scientific literature, the EPA could not find evidence of *E. coli* strain K-12 enterotoxin-induced disease related to occupational exposure (U.S. EPA, 1997a). The EPA therefore concluded that *E. coli* strain K-12 has a history of safe use. *E. coli* strain K-12 is included in the list of Tier I exempt microorganisms under the *Microbial Products of Biotechnology Final Regulation Under the Toxic Substances Control Act* (U.S. EPA, 1997a).

Moreover, the WGS of Tate & Lyle's production organism (GM *E. coli* strain K-12) was subject to sequence homology searches to identify genes encoding for virulence factors and bacterial exotoxins. To identify genes involved in toxigenicity, a sequence homology search was conducted using BLAST (with the WGS of the production organism) and the Database of Bacterial Exotoxins for Humans. The pathogenic potential of *E. coli* strain K-12 was also assessed *via* sequence homology searches using BLAST against the Virulence Factor Database. Sequence alignments sharing ≥80% identity and ≥70% query coverage were considered to share significant sequence homology. Results of the analyses are presented in Appendix A-4 **[CONFIDENTIAL]**. All identified genes with significant sequence homology to a bacterial exotoxin or virulence factor are present in the genome of *E. coli* strain K-12, a well-characterised strain with a documented history of safe use in the production of enzymes for use in food production. The expression plasmid used for the construction of the production organism has been fully sequenced and does not possess genetic elements of concern, with the exception of an antimicrobial resistance (AMR) gene (see Section B.6.2). The gene encoding the D-psicose 3-epimerase enzyme was synthesised to ensure that no unidentified extraneous DNA from the donor organism was transferred to the production organism. Taken together, it can be concluded that the genetic modification steps to construct the production organism did not result in the introduction of genes encoding bacterial exotoxins or virulence factors. The pathogenic and toxigenic potential of the production organism is considered to be low.

### D.2.2 Antimicrobial Resistance

The WGS of Tate & Lyle's production organism was analysed for AMR genes using 2 manually curated databases of AMR genes: ResFinder and AMRFinder. Sequence homology searches were conducted using BLAST. Sequence alignments sharing ≥80% identity and ≥70% query coverage were considered to share significant sequence homology. Results of the analyses are presented in Appendix A-4 **[CONFIDENTIAL]**. All identified genes with significant sequence homology to an AMR gene are naturally present in the genome of *E. coli* strain K-12, with the exception of one gene conferring resistance to chloramphenicol identified on the expression plasmid. The gene was used during the development of the production organism as a selection marker on the expression plasmid. However, this AMR gene is not expected to pose a safety concern. The manufacturing process of D-psicose 3-epimerase incorporates steps to ensure the removal of residual DNA from the production organism. The absence of residual DNA from the production organism has been demonstrated in the enzyme (see Section C.2.4). As such, there is no risk of horizontal gene transfer of AMR genes to other bacterial species or the environment.

### D.2.3 Absence of the Production Strain

The absence of viable cells of the production organism (GM *E. coli* strain K-12) in D-psicose 3-epimerase was confirmed analytically in an experiment conducted according to the recommendations set out in EFSA's *Scientific Guidance for the Submission of Dossiers of Food Enzymes* (EFSA, 2021b). The full details of the methodology are provided in Appendix A **[CONFIDENTIAL]**, and the study report is provided in Appendix A-5 **[CONFIDENTIAL]**.

Three non-consecutive batches of D-psicose 3-epimerase enzyme produced using the industrial-scale manufacturing process were collected for analysis. Test samples were inoculated on culture plates. Positive and spiked control plates were prepared with low counts of viable cells of the production strain to demonstrate normal growth of the production strain. All plates were visually examined for colonies. No viable cells were detected in the cultures of the 3 batches of D-psicose 3-epimerase. Cell growth was observed in the positive control plates, confirming that the medium and cultivation conditions were suitable for cell growth.

### D.2.4 Absence of Residual DNA

The absence of residual DNA from the production organism (GM *E. coli* strain K-12) in D-psicose 3-epimerase was confirmed analytically in an experiment conducted according to the recommendations set out in EFSA's *Scientific Guidance for the Submission of Dossiers of Food Enzymes* (EFSA, 2021b). The full details of the methodology are provided in Appendix A **[CONFIDENTIAL]**, and the study report is provided in Appendix A-6 **[CONFIDENTIAL]**. Three non-consecutive batches of D-psicose 3-epimerase produced using the industrial-scale manufacturing process were analysed *via* polymerase chain reaction analysis. No residual DNA from the production organism was detected in any of the tested batches.

## D.3 Information on the Genetic Stability of the Source Organism

The source organism is a GM strain of *E. coli* strain K-12. The production organism is stored at a recognised culture collection until needed and is maintained in a microbial collection at -80°C at the production facility. Tate & Lyle maintains a well-defined cell bank system using a master cell bank and working cell bank to store the production strain.

The production process of D-psicose 3-epimerase complies with HACCP principles and incorporates quality control steps throughout the production process, including the fermentation steps, to ensure that specific criteria are met. Batches that do not meet the specifications for the enzyme are not released for further processing.

# E. INFORMATION RELATED TO THE SAFETY OF AN ENZYME PROCESSING AID DERIVED FROM A GENETICALLY MODIFIED MICROORGANISM

In accordance with Part E of Section 3.3.2, Processing aids, of the *Food Standards Australia New Zealand Application Handbook* (FSANZ, 2025), the following safety information must be provided for enzyme processing aids:

1. Information on the methods used in the genetic modification of the source organism

Each point is addressed in the following subsections.

## E.1 Information on the Methods Used in the Genetic Modification of the Source Organism

The *E. coli* genome does not contain the gene encoding the D-psicose 3-epimerase enzyme. The parental strain, *E. coli* strain K-12, was genetically modified in order to construct a production strain that expressed D-psicose 3-epimerase. To this end, a synthetic DNA construct for the gene based on the native gene from *Desmospora* sp. 8437 was designed and codon-optimised for expression in *E. coli*. The genetic construct was inserted into a commercial plasmid vector. No modifications were made to the plasmid vector outside of the introduction of the gene encoding for D-psicose 3-epimerase. The synthetic gene was demonstrated to share a high degree of sequence similarity with the native gene from the *Desmospora* sp. 8437. A map of the expression plasmid is provided in Appendix A-4. The expression plasmid containing the gene for D-psicose 3-epimerase was introduced to *E. coli* strain K-12 *via* electroporation. Molecular characterization of the production organism was performed using WGS, which confirmed that the expression plasmid containing the gene encoding for D-psicose 3-epimerase was not integrated into the genome of the production organism. Further information about the construction of the production strain is provided in Appendix A **[CONFIDENTIAL]**.

# F. INFORMATION RELATED TO THE DIETARY EXPOSURE TO THE PROCESSING AID

In accordance with Part F of Section 3.3.2, Processing aids, of the *Food Standards Australia New Zealand Application Handbook* (FSANZ, 2025), the following dietary exposure information must be provided:

- A list of foods or food groups likely to contain the processing aid or its metabolites;
- The levels of residues of the processing aid or its metabolites for each food or food group;
- For foods or food groups not currently listed in the most recent Australian or New Zealand National Nutrition Surveys (NNSs), information on the likely level of consumption;
- The percentage of the food group in which the processing aid is likely to be found or the percentage of the market likely to use the processing aid;
- Information relating to the levels of residues in foods in other countries; and
- For foods where consumption has changed in recent years, information on likely current food consumption.

Each point is addressed in the following subsections.

## F.1 A List of Foods or Food Groups Likely to Contain the Processing Aid or Its Metabolites

D-psicose 3-epimerase is intended for use in the production of D-allulose at a maximum use level of up to 12.6 mg TOS/kg in D-fructose as a raw material. A dietary exposure assessment for D-psicose 3-epimerase from GM *E. coli* strain K-12 was not conducted because of the absence of enzyme TOS demonstrated in the final D-allulose product (see Section B.3.3.4).

## F.2 The Levels of Residues of the Processing Aid or Its Metabolites for Each Food or Food Group

During the production of D-allulose, the immobilised D-psicose 3-epimerase enzyme is added to D-fructose syrup at the start of the food manufacturing process. Following the enzymatic reaction, the crude D-allulose syrup is filtered then subjected to separation, purification, and refinement processes to remove proteins and other substances. These purification processes effectively remove enzyme TOS from the final D-allulose product. The removal of enzyme TOS was demonstrated analytically by measuring the protein content of 3 batches of the final D-allulose syrup using the Bradford assay. No residual protein was detected in the tested batches, demonstrating the absence of enzyme TOS in the final product. As a result, a dietary exposure assessment of the D-psicose 3-epimerase was not considered necessary.

## F.3 For Foods or Food Groups Not Currently Listed in the Most Recent Australian or New Zealand National Nutrition Surveys (NNSs), Information on the Likely Level of Consumption

Not applicable.

## F.4 The Percentage of the Food Group in Which the Processing Aid is Likely To Be Found or the Percentage of the Market Likely to Use the Processing Aid

D-psicose 3-epimerase from *M. foliorum* is currently approved for use in Australia and New Zealand per Schedule 18 of the Code (FSANZ, 2026). Tate & Lyle's D-psicose 3-epimerase from GM *E. coli* strain K-12 is anticipated to provide food manufacturers and producers with an alternative processing aid for the production of D-allulose . Therefore, approval for D-psicose 3-epimerase from GM *E. coli* strain K-12 is not expected to increase the existing use of D-psicose 3-epimerase in food production.

## F.5 Information Relating to the Levels of Residues in Foods in Other Countries

As discussed in Section A.3.3.4, no enzyme residues are expected during the production of D-allulose given the purification steps involved in the production process. Marginal amounts of enzyme residue carryover would not pose any safety concerns because of the high margins of safety determined from the safety data presented in Section B.2.2.

## F.6 For Foods Where Consumption Has Changed in Recent Years, Information on Likely Current Food Consumption

D-psicose 3-epimerase enzyme is intended for use in the production of D-allulose, which can in turn be added to a variety of food products such as bakery products, bubble gum, breakfast cereals, cereal bars, chewing gums, confectionary, desserts, edible ices, frostings, fruit spreads, ice cream, icings, imitation cream, non-alcoholic water-based flavoured drinks, salad dressings, sweet sauces, syrups, tabletop sweeteners, and yoghurt. It is not anticipated that the intakes of these food products have drastically changed in recent years.

# REFERENCES


Anderson ES (1975). Viability of, and transfer of a plasmid from, *E. coli* K12 in human intestine. Nature 255(5508):502-504. DOI:10.1038/255502a0. Cited In: U.S. EPA, 1997b.

Archer CT, Kim JF, Jeong H, Park JH, Vickers CE, Lee SY, et al. (2011). The genome sequence of *E. coli* W (ATCC 9637): comparative genome analysis and an improved genome-scale reconstruction of *E. coli*. BMC Genomics 12:9 [20pp]. DOI:10.1186/1471-2164-12-9.

Bauer AP, Ludwig W, Schleifer KH (2008). A novel DNA microarray design for accurate and straightforward identification of *Escherichia coli* safety and laboratory strains. Syst Appl Microbiol 31(1):50-61. DOI:10.1016/j.syapm.2008.01.001.

Codex Alimentarius (2003). *Guideline for the Conduct of Food Safety Assessment of Foods Produced Using Recombinant-DNA Microorganisms*. (CAC/GL 46-2003; Adopted in 2003, Annexes II and III adopted in 2008). Rome Italy: Codex Alimentarius. Available at: https://www.who.int/docs/default-source/food-safety/food-genetically-modified/cxg-046e.pdf.

Codex Alimentarius (2009). *Foods Derived from Modern Biotechnology, 2nd edition*. Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) / Geneva, Switzerland: World Health Organization (WHO), Codex Alimentarius Commission. Available at: https://www.fao.org/4/a1554e/a1554e00.htm.

EFSA (2017). Guidance on allergenicity assessment of genetically modified plants (EFSA Panel on Genetically Modified Organisms/GMO) (Question no: EFSA-Q-2014-00547, adopted: 18 May 2017 by European Food Safety Authority). EFSA J 15(6):4862 (49pp). DOI:10.2903/j.efsa.2017.4862. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/4862.

EFSA (2021a). Scientific Opinion on the safety evaluation of the food enzyme D-psicose 3-epimerase from the genetically modified *Escherichia coli strain* K-12 W3110 (pWKLP) (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids/CEP) (Question no: EFSA-Q-2019-00445; adopted 24 March 2021 by European Food Safety Authority). EFSA J 19(4):6565 (19pp). DOI:10.2903/j.efsa.2021.6565. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/6565.

EFSA (2021b). Scientific Guidance for the submission of dossiers of Food Enzymes (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids/CEP) (Question no: EFSA-Q-2020-00477, adopted: 15 September 2021. Appendix D updated on 7 June 2023 by European Food Safety Authority). EFSA J 19(10):6851 (35pp). DOI:10.2903/j.efsa.2021.6851. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/6851.

EFSA (2024). EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain (Question no: EFSA-Q-2022-00250 adopted: 28 June 2024, by European Food Safety Authority). EFSA J 22(8):8912 (14pp). DOI:10.2903/j.efsa.2024.8912. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/8912.

EFSA (2025). Safety evaluation of the food enzyme D-psicose 3-epimerase from the genetically modified *Escherichia coli* strain K-12 W3110-TKO CDX-088 (EFSA Panel on Food Enzymes/FEZ) (Question no: EFSA-Q-2024-00310; adopted: 11 September 2025 by European Food Safety Authority). EFSA J 23(10):9666 (10pp). DOI:10.2903/j.efsa.2025.9666. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/9666.

FAO/WHO (2001). *Evaluation of Allergenicity of Genetically Modified Foods*. Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology, 22-25 January 2001. Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) / Geneva, Switzerland: World Health Organization (WHO). Available at: https://www.who.int/publications/m/item/evaluation-of-allergenicity-of-genetically-modifieD-foodsreport-of-a-joint-fao-who-expert-consultation-on-allergenicity-of-foods-deriveD-from-biotechnology.

FCC (2026). Enzyme preparations. In: *Food Chemicals Codex, 14th edition [online].* Rockville (MD): United States Pharmacopeial Convention [effective date: 01-Jun-2015]. Available at: https://www.foodchemicalscodex.org/ (subscription required).

FSANZ (2025). *Food Standards Australia New Zealand Application Handbook*. Canberra, Australia / Wellington, NZ: Foods Standards Australia New Zealand (FSANZ). Available at: https://www.foodstandards.gov.au/food-standards-code/changing-the-code/applicationshandbook (1 October 2025).

FSANZ (2026). Schedule 18 — Processing aids (F2026C00200). In: *Australia New Zealand Code*. Canberra, Australia / Wellington, NZ: Foods Standards Australia New Zealand (FSANZ), Australian Government. Available at: https://www.legislation.gov.au/F2015L00452/latest/text.

IFBC (1990). Chapter 4: Safety evaluation of foods and food ingredients derived from microorganisms (International Food Biotechnology Council - IFBC). Regul Toxicol Pharmacol 12(3, Part 2):S114-S128. DOI:10.1016/S0273-2300(05)80080-7.

JECFA (2006a). General specifications and considerations for enzyme preparation used in food processing [prepared by the Committee at its sixty-seventh meeting (2006)]. In: *Combined Compendium of Food Additive Specifications [Online Edition]. Volume 4: Analytical Methods, Test Procedures and Laboratory Solutions Used by and Referenced in the Food Specifications*. 1st to 67th JECFA Meetings, 1956-2006. (FAO JECFA Monographs No. 1). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) / Geneva, Switzerland: Joint FAO/WHO Expert Committee on Food Additives (JECFA), xxi-xxv. Available at: https://www.fao.org/3/a0691e/a0691e00.htm & http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/enzymes/en/.

JECFA (2006b). Antibacterial activity. In: *Combined Compendium of Food Additive Specifications [Online Edition]. Volume 4: Analytical Methods, Test Procedures and Laboratory Solutions Used by and Referenced in the Food Specifications*. 1st to 67th JECFA Meetings, 1956-2006. (FAO JECFA Monographs No. 1). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) / Geneva, Switzerland: Joint FAO/WHO Expert Committee on Food Additives (JECFA), 122-123. Available at: https://www.fao.org/3/a0691e/a0691e00.htm.

JECFA (2020). (Framework for) Steviol glycosides. In: *Combined Compendium of Food Additive Specifications*. 87th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), June 4-13, 2019, Rome, Italy. (FAO JECFA Monographs, No. 23). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) / Geneva, Switzerland: World Health Organization (WHO), pp. 62-85. Available at: https://openknowledge.fao.org/items/fed24264-6796-4dec-b35D-0ee1e157b8cc.

JECFA (2021). (Framework for) Steviol glycosides [Prepared at the 91st JECFA, superseding specifications prepared at the 87th JECFA]. In: *Combined Compendium of Food Additive Specification.* 91st Virtual Meeting of JECFA, Feb. 1-12, 2021. (FAO JECFA Monographs, No. 26). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) / Geneva, Switzerland: Joint FAO/WHO Expert Committee on Food Additives (JECFA), pp. 3-32, 46. Available online: https://openknowledge.fao.org/items/4531ccdf-4945-4a54-92cf-47017f4d2380.

Jeong H, Barbe V, Lee CH, Vallenet D, Yu DS, Choi SH, et al. (2009). Genome sequences of *Escherichia coli* B strains REL606 and BL21(DE3). J Mol Biol 394(4):644-652 [plus supplementary data]. DOI:10.1016/j.jmb.2009.09.052.

Jürkenbeck K, Haarhoff T, Spiller A, Schulze M (2022). Does allulose appeal to consumers? Results from a discrete choice experiment in Germany. Nutrients 14(16):3350 [12pp]. DOI:10.3390/nu14163350.

Levy SB, Marshall B, Rowse-Eagle D, Onderdonk A (1980). Survival of *Escherichia coli* host-vector systems in the mammalian intestine. Science 209(4454):391-394. DOI:10.1126/science.6992276. Cited In: U.S. EPA, 1997b.

NIH (2024). *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*. (NIH Guidelines). Bethesda (MD): National Institutes of Health (NIH), Office of Science Policy. Available at: https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf (April 2024).

OECD (1992). *Safety Considerations for Biotechnology 1992*. Paris, France: Organisation for Economic Co-Operation and Development (OECD). Available at: https://web.archive.org/web/20201030014308/https://www.oecd.org/sti/emerging-tech/2375496.pdf.

Pariza MW, Foster EM (1983). Determining the safety of enzymes used in food processing. J Food Prot 46(5):453-468. DOI:10.4315/0362-028X-46.5.453.

Pariza MW, Johnson EA (2001). Evaluating the safety of microbial enzyme preparations used in food processing: update for a new century. Regul Toxicol Pharmacol 33(2):173-186. DOI:10.1006/rtph.2001.1466.

Smith HW (1975). Survival of orally administered *E. coli* K 12 in alimentary tract of man. Nature 255(5508):500-502. DOI:10.1038/255500a0. Cited In: U.S. EPA, 1997b.

U.S. EPA (1997b). *Escherichia Coli K-12 Final Risk Assessment: Attachment I--Final Risk Assessment of Escherichia Coli K-12 Derivatives*. Washington (DC): U.S. Environmental Protection Agency (U.S. EPA), Biotechnology Program under the Toxic Substances Control Act (TSCA). Available at: https://www.epa.gov/sites/production/files/2015-09/documents/fra004.pdf (last updated on September 27, 2012).

U.S. EPA (1997a). Microbial products of biotechnology; Final Regulation Under the Toxic Substances Control Act. (40 CFR Parts 700, 720, 721, 723, and 725) [OPPTS–00049C; FRL–5577–2; RIN 2070-AB61]. Fed Regist (US) 62(70):17910-17958. Available at: https://www.federalregister.gov/documents/1997/04/11/97-8669/microbial-products-of-biotechnology-final-regulation-under-the-toxic-substances-control-act.

U.S. FDA (2016). *Agency Response Letter GRAS Notice No. GRN 624 [D-allulose 3-epimerase from Arthrobacter globiformis M30 produced in Escherichia coli, Itami City, Hyogo, Japan: Matsutani Chemical Industry Co. Ltd*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.hfpappexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=624 (Aug. 18, 2016 - FDA response - no questions).

UniProt Consortium (2025). UniProt: the Universal Protein Knowledgebase in 2025. Nucleic Acids Res 53(D1):D609-D617. DOI:10.1093/nar/gkae1010.

UniProt Consortium (2026). UniProtKB [search terms: ((cc_tissue_specificity:venom) OR (keyword:KW 0800)) AND (reviewed:true)]. In: *UniProt: the Universal Protein Knowledgebase*. Geneva, Switzerland: UniProt Consortium (Universal Protein). Available at: https://www.uniprot.org/ & https://www.uniprot.org/uniprotkb?query=KW-0800 (©2002-2026).

Xia Y, Cheng Q, Mu W, Hu X, Sun Z, Qiu Y, et al. (2021). Research advances of D-allulose: an overview of physiological functions, enzymatic biotransformation technologies, and production processes. Foods 10(9):2186 [21pp]. DOI:10.3390/foods10092186.