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

24 November 2026
422-26

Supporting document 1

Risk and technical assessment – Application A1354

D-psicose 3-epimerase from *Escherichia coli* K-12 (gene donor: *Desmospora sp.* 8437*)* for use as a processing aid

Executive summary

Tate and Lyle Solutions USA LLC has applied to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of the immobilised enzyme D-psicose 3-epimerase (EC 5.1.3.30) from *Escherichia coli* K-12 containing the D-psicose 3-epimerase gene from *Desmospora* sp. 8437. The enzyme will be used as a processing aid to produce D-allulose. It catalyses the epimerisation of D-fructose to D-allulose, a low-energy sugar substitute for conventional sugar ingredients.

The available evidence provides adequate assurance the proposed use of immobilised D-psicose 3-epimerase from *Desmospora* sp. 8437 as a processing aid is technologically justified. Immobilised D-psicose 3-epimerase performs its technological function during food processing and, as such, meets the definition of a processing aid for the purposes of the Code.

The amino acid sequence of the D-psicose 3-epimerase has no homology with any known toxin or allergen, and there are no case reports of allergic reactions to the enzyme. The enzyme is used when immobilised on a food-grade resin and cannot be detected in the final D-allulose product. Therefore the likelihood of consumer exposure to the enzyme is negligible. For this reason, FSANZ concludes that toxicological studies are not necessary, and derivation of an Acceptable Daily Intake (ADI) is not required.

No public health or safety concerns were identified concerning the use of the production organism, which is neither pathogenic nor toxigenic. Analysis of the production strain confirmed the presence and stability of the inserted DNA.

FSANZ has not undertaken a dietary exposure assessment for the enzyme because analytical testing confirmed that it is unlikely to be present in the final D-allulose products and there is no ADI. This is consistent with similar assessments conducted by FSANZ and internationally.

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1 Introduction

Tate and Lyle Solutions USA LLC applied to Food Standards Australia New Zealand (FSANZ) to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of the immobilised enzyme D-psicose 3-epimerase (EC 5.1.3.30) from *Escherichia* coli K-12 containing the D-psicose 3-epimerase gene from *Desmospora* sp. 8437 as a processing aid.

Immobilised D-psicose 3-epimerase will be used to catalyse the epimerisation of D-fructose for the large- scale production of D-allulose, a low-energy sugar substitute for conventional sugar ingredients, particularly sucrose. It will be used at the minimum level required to achieve the desired effect, in accordance with the principles of Good Manufacturing Practice (GMP)[1].

1.1 Objectives of the assessment

The objectives of this risk and technical assessment were to:

- determine whether the proposed purpose is solely a technological function and that the enzyme 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 enzyme as a processing aid by considering the:
  - safety and history of use of the production microorganism
  - safety of the enzyme preparation.

2 Food technology assessment

2.1 Identity of the enzyme

The applicant provided information regarding the identity of the enzyme and this has been verified using the International Union of Biochemistry and Molecular Biology (IUBMB) enzyme nomenclature reference database (McDonald et al. 2009). Details of the identity of the enzyme are provided below.

Accepted IUBMB name:	D-psicose 3-epimerase
Systematic name:	D-psicose 3-epimerase
Other names	D-allulose 3-epimerase, DPEase (ambiguous), DTEase
IUBMB enzyme nomenclature:	EC 5.1.3.30
CAS number:	1219591-85-1

[1] GMP is defined in section 1.1.2—2 of the Code as follows: ***GMP*** *or* ***Good Manufacturing Practice****, with respect to the addition of substances used as food additives and substances used as processing aids to food, means the practice of:*
(a) limiting the amount of substance that is added to food to the lowest possible level necessary to accomplish its desired effect; and
(b) to the extent reasonably possible, reducing the amount of the substance or its derivatives that:
*(i) remains as a *component of the food as a result of its use in the manufacture, processing or packaging; and*
(ii) is not intended to accomplish any physical or other technical effect in the food itself;
(c) preparing and handling the substance in the same way as a food ingredient.

Reaction: D-fructose = D-psicose

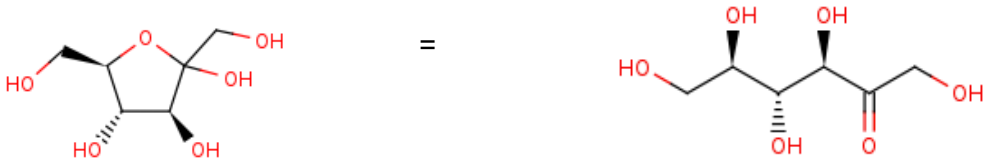

Figure 1: Reaction catalysed by D-psicose 3-epimerase (Source: BRENDA)

2.2 Manufacturing process

2.2.1 Production of the enzyme

Enzymes from microorganisms are typically produced by controlled fermentation followed by removal of the production microorganism, purification and concentration of the 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 a specific reaction during food processing or two or more active enzymes that catalyse different reactions (FAO/WHO 2020).

D-psicose 3-epimerase is produced by fermentation of *Escherichia coli* K-12. The fermentation process comprises three operations: direct inoculation of the stock culture suspension into the seed fermenter, seed fermentation and main fermentation, the latter of which is operated under submerged fed-batch conditions.

Once fermentation is complete, a recovery process involving multiple steps to separate the *E. coli* biomass from the enzyme-containing culture medium is undertaken. This includes centrifugation and cell lysis. The supernatant is then filtered to remove residual DNA, followed by ultra-filtration and freeze-drying to stabilise the enzyme. The enzyme is immobilised on a food-grade ion-exchange resin which is then used for the epimerisation of D-fructose to D-allulose. Subsequent separation, purification, and refinement steps eliminate any residual proteins and particles of the immobilisation resin.

The applicant has stated the enzyme is manufactured in accordance with GMP and the principles of Hazard Analysis of Critical Control Points and has provided evidence of compliance of the production site with the Food Safety System Certification 22000 (FSSC 22000) standard. The resultant product meets the general specifications for enzyme preparations used in food processing as established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Food Chemicals Codex (FCC) (see also section 2.2.2 of this report).

Information on the raw materials used in the production and recovery of the enzyme preparation is deemed confidential commercial information (CCI) under section 114 of the *Food Standards Australia New Zealand Act 1991* (the FSANZ Act). This information has been evaluated by FSANZ but cannot be disclosed in this report.

2.2.2 Specifications for identity and purity

There are international general specifications for enzyme preparations used in the production of food, established by JECFA in its Compendium of Food Additive Specifications and in the FCC (13th edition), referenced in section S3—2 of Schedule 3 of the Code. Enzymes used as processing aids need to meet either of these specifications, or a relevant specification in section S3—3 of Schedule 3.

Schedule 3 of the Code 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 applicant provided analytical data for three representative standardised batches of the enzyme. Based on those results the enzyme met the relevant specifications in the Code.

2.3 Technological purpose

The D-psicose 3-epimerase enzyme preparation will be used as a processing aid to catalyse the reversible epimerisation of D-fructose at the C-3 position, in the presence of a divalent metal ion cofactor to yield D-allulose (Kim et al. 2006). D-allulose is monosaccharide sugar that is used as a low-energy sugar substitute for conventional sugar ingredients, particularly sucrose.

Table 1: D-psicose 3-epimerase enzyme preparation physical/chemical properties

Physical/chemical properties of commercial enzyme preparation	
Enzyme activity	>80 U/mg*
Appearance	Lyophilised white to coloured powder
Temperature optimum	75°C, with activity decreasing above or below this temperature
Thermostability	Enzyme activity decreases above and below 75 °C
pH range and optimum	Range pH 7.4-10, peaking at pH 10

*U = enzyme activity units, where one unit is the amount of enzyme that isomerise 1 micromole of D-fructose under the conditions of the assay

2.4 Allergen considerations

The applicant provided confidential product information which indicated the enzyme preparation does not contain known food allergens.

2.5 Food technology conclusion

The use of immobilised D-psicose 3-epimerase to catalyse the epimerisation of D-fructose to D-allulose is consistent with its functions as a processing aid.

The evidence presented to support its proposed use in the production of D-allulose provides adequate assurance that the use of the enzyme, in the quantity and form proposed to be used (which must be consistent with GMP), is technologically justified.

Immobilised D-psicose 3-epimerase performs its technological purpose during the manufacture of food products and the applicant has provided evidence that it is not present in the final food. This is due to the immobilisation of the enzyme on an ion exchange resin. It therefore functions 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 has provided evidence that the enzyme meets these specifications.

3 Safety assessment

This safety assessment aims to evaluate potential public health and safety concerns that may arise from using this enzyme as a processing aid.

Some information relevant to this section is deemed CCI under section 114 of the FSANZ Act. This information has been evaluated by FSANZ but cannot be disclosed in this report.

3.1 Source microorganism

The production strain used for the manufacture of D-psicose 3-epimerase was derived from *Escherichia coli* K-12 W3110-TKO. W3110-TKO is a substrain of *E. coli* K-12 with well-characterised genetic divergence from the K-12 parent strain (Hayashi et al. 2006). Accordingly, FSANZ's microbiological assessment focuses on the safety of the parental *E. coli* K-12 strain.

E. coli is a facultatively anaerobic, gram-negative, rod-shaped bacterium found in the gut of mammals (Guerra et al. 2019). Some *E. coli* strains can be pathogenic to humans causing a wide range of diseases, which can be fatal (Guerra et al. 2019, Mir and Kudva 2019). However, there are strains of *E. coli*, termed safe strains, that are used in research and industry specifically because of their inability to cause disease in humans (Bauer et al. 2008). K-12 is one of these safe strains and lacks well-recognised pathogenic mechanisms associated with disease and is the most common bacterial laboratory strain in use globally.

Comparative genome sequencing and proteomic analyses comparing *E. coli* K-12 and its derivatives with well-characterised pathogenic strains have identified several features associated with the reduced pathogenic potential of K-12 (Bachmann 1996; EPA 1997; Sahl et al. 2013). These include alterations to cell wall structure that reduce colonisation of the human intestinal tract, as well as the absence of adhesive proteins and key virulence factors required for pathogenicity. These studies also demonstrated reduced toxin production and the absence of plasmids encoding antibiotic resistance determinants. Under the U.S. National Institutes of Health Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH, 2024), *E. coli* K-12 is classified as a Risk Group 1 organism, a category reserved for agents designated as non-pathogenic for both human and animals.

E. coli K-12 has a long history of use in biopharmaceutical manufacturing, with approximately 30% of recombinant therapeutic proteins approved in the United States produced using *E. coli* K-12 expression systems (Huang et al. 2012; Jozala et al. 2016). The use of this bacterium as a source for the production of food enzymes began in the 1980s (JECFA 1991). *E. coli* K-12 is a model organism that has been extensively characterised for research and industrial applications and is therefore regarded as safe for these purposes. Given its extensive history of industrial use, *E. coli* K-12 has been evaluated by multiple regulatory agencies, including the U.S. Food and Drug Administration, the European Food Safety Authority (EFSA), and FSANZ (applications A1233, A1268, and A1277), for use in industrial production processes conducted under GMP.

The taxonomic identity of the production strain was confirmed by whole-genome sequencing (WGS) and bioinformatic analysis. In addition, batch analyses demonstrate that the final product does not contain viable cells from the production organisms.

The microbiology assessment identified no public health and safety concerns regarding the use of *E. coli* K-12 derived strains as production organisms.

3.2 Characterisation of the genetic modification to the production strain

3.2.1 Description of the DNA to be introduced and the method of transformation

A plasmid vector containing the D-psicose 3-epimerase gene was introduced into the host *E. coli* K-12 using standard molecular biology techniques. The D-psicose 3-epimerase gene was synthesised *in vitro* based on the native DNA sequence from *Desmospora* sp. 8437 available in public databases, and codon optimised for expression in the host.

Data provided by the applicant and analysed by FSANZ confirmed the identity of the D-psicose 3-epimerase gene.

3.2.2 Characterisation of the introduced DNA

The plasmid vector is episomally maintained and is not integrated into the host genome. WGS analysis confirmed the presence of the plasmid carrying the D-psicose 3-epimerase gene and demonstrated the absence of plasmid-derived DNA in the production strain genome.

3.2.3 Stability of the introduced DNA

The applicant provided protein expression and functional data across multiple production batches as a proxy of the stability of the introduced DNA. Total protein was assessed by SDS-PAGE, which showed a consistent protein band corresponding to the expected molecular mass of the enzyme across all batches analysed. Similarly, the enzymatic activity of D-psicose 3-epimerase was consistent across all batches analysed.

Together, these data confirm the stability of the expression plasmid in the production strain over multiple generations.

3.3 Safety of the enzyme

3.3.1 History of safe use

There is permission for D-psicose 3-epimerase from a difference source in the Code.

3.3.2 Bioinformatic assessment of homology with known toxins

A recent BLAST search for homology of the D-psicose 3-epimerase sequence against the complete UniProt[2] database was performed, using criteria for significance of ≥80% identity and ≥70% coverage. No significant homology with any known toxin or venom was found.

3.3.3 Toxicology data

The likelihood of residual D-psicose 3-epimerase being present in the final D-allulose is low. The enzyme is intended for use only when immobilized on a food-grade resin, and confidential data were supplied that indicate that the enzyme has been shown to be below the limit of detection (LOD) of 3.4 mg/kg in multiple batches of the final D-allulose.

Given the enzyme is intended to be used in immobilized form and not detected in D-allulose. an Acceptable Daily Intake (ADI) is not required.

3.3.4 Potential for allergenicity

To investigate the possibility of allergenic cross-reactivity of this D-psicose 3-epimerase,

[2] https://www.uniprot.org/

recent searches were conducted against the AllergenOnline database (Version 23) using a default E-value of 1:

- a sliding window of 80–amino acid sequences derived from each full-length amino acid sequence of the enzyme
- an 8–amino acid exact match
- the full-length amino acid sequence.

No matches of greater than 35% identity were identified in any of the searches.

The application also included the results of a thorough literature search of numerous databases. No allergenicity of D-psicose 3-epimerase was further considered through a search of available scientific literature. No evidence of allergic responses to oral or inhalation exposure to the enzyme were located.

3.3.5 Assessments by other regulatory agencies

The D-psicose 3-epimerase that is the subject of this application has been evaluated by the 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, the FEZ Panel waived toxicological testing requirements. The Panel concluded that the enzyme does not give rise to safety concerns under the intended conditions of use (EFSA, 2025).

4 Dietary exposure assessment

Due to the proposed manufacturing process including purification steps outlined in sections 2.2.1 and 3.3.3, residual D-psicose 3-epimerase is unlikely to be present in the final D-allulose products, as confirmed by analytical testing. Therefore, consumer exposure to the enzyme through consumption of D-allulose manufactured using this process is expected to be negligible. On this basis, FSANZ has not undertaken a dietary exposure assessment for D-psicose 3-epimerase. This approach is consistent with the EFSA (2025) assessment for the subject of this application and FSANZ's similar assessment for A1247 (2024).

5 Discussion

The use of immobilised D-psicose 3-epimerase from *Escherichia* coli K-12 containing the gene from *Desmospora* sp. 8437 as a processing aid in the production of D-allulose is consistent with its known function. It will be used to catalyse the epimerisation of D-fructose to D-allulose. The enzyme is intended to be used in the large-scale production of D-allulose, a low-energy sugar substitute for conventional sugar ingredients, particularly sucrose.

Immobilised D-psicose 3-epimerase is functioning as a processing aid for the purposes of the Code and does not perform a technological purpose in the food for sale. The evidence presented to support its proposed use provides adequate assurance that the use of the enzyme, in the quantity and form proposed to be used (which must be consistent with GMP), is technologically justified.

No public health or safety concerns were identified concerning the use of the production organism, which is neither pathogenic nor toxigenic. Analysis of the production strain confirmed the presence and stability of the inserted DNA.

No significant homology between the enzyme and any known toxins or allergens was identified.

The enzyme cannot be detected in the final D-allulose product. Therefore, the likelihood of consumer exposure to the enzyme is negligible. For this reason, FSANZ concludes that toxicological studies are not necessary, and derivation of an ADI is not required. Therefore, a dietary exposure assessment was not undertaken.

FSANZ concludes there are no safety concerns from the use of immobilised D-psicose 3-epimerase from *Escherichia* coli K-12 using the D-psicose 3-epimerase gene from *Desmospora* sp. 8437 to catalyse the epimerisation of D-fructose to D-allulose in the quantity and form required to perform its typical function, which must be consistent with GMP.

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