A Xylanase Enzyme from a recombinant strain of *Trichoderma reesei*

PROCESSING AID APPLICATION

Food Standards Australia New Zealand

Applicant: IFF Australia Pty Ltd (Trading as Danisco Australia Pty Ltd)

31st October 2022

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1. General Information

1.1 Applicant details

(a) Applicant:
This application is made by Danisco Australia (IFF)

[redacted]

(e) Email address:
See above

(f) Nature of Applicants Business:
Danisco Australia Pty Ltd – A subsidiary of International Flavors and Fragrances Inc (IFF), manufacturer/marketer of specialty food ingredients, food additives and food processing aids. Danisco Australia is also an affliate of Genencor International Ltd, the manufacturer of the prodcut and another subsidiary of International Flavors and Fragrances Inc (IFF). Entity Relationship letter, Section 1.2.

(g) Details of Other Individuals.:
No other individuals, companies or organisations are associated with this application.

1.2 Entity Relationship letter

September 11, 2022

Where science
& creativity meet

Re: Statement Regarding Subsidiary Entities

To Whom It May Concern,

We are pleased to inform you that on February 1, 2021, International Flavors & Fragrances Inc. ("IFF") completed its previously announced combination of IFF and the Nutrition & Biosciences business (the "N&B Business") of DuPont de Nemours, Inc. ("DuPont") (the "Merger").

As part of the Merger, various legal entities associated with the N&B business, were transferred by DuPont to IFF. The N&B legal entities listed on the following website are among the legal entities associated with the N&B business that were transferred by DuPont to IFF.

https://www.iff.com/where-we-operate/subsidiaries

For your purposes, please consider the following N&B legal entities as operating under IFF ownership as IFF Health & Biosciences business.

[redacted]

[redacted]

Thank you for your attention.

Sincerely,

[redacted]

[redacted]

1.3 Purpose of the Application

This application seeks to modify Schedule 18 to Standard 1.3.3 Processing Aids to permit the use of a new Processing Aid, subject of this application. The intended use of the processing aid is starch processing and potable alcohol production.

This application is made solely on behalf of Dansico Australia (henceforth referred to as IFF throughout the dossier), the manufacturer/marketer of the Processing Aid. When approved, the Processing Aid would be available for use by any food manufacturer in Australia and New Zealand.

Xylanase, subject of this application, is intended for use for the production of potable alcohol and in starch processing.

Currently no Xylanase from *Fusarium verticillioides* expressed in *T. reesei* is permitted as a Processing Aid, however Xylanase from *T. reesei*, and other enzymes including Cellulase, Endo-1,4-beta-xylanase, β-Glucanase, Polygalacturonase, Hemicellulase multicomponent enzyme, or Pectinase multicomponent enzyme, from *T. reesei* are listed in Schedule 18 section S18-4(5) as permitted enzymes. Approval of this application would provide food processors with a new enzyme preparation offering the benefits and advantages as discussed in Section 2.3 and Appendix A.

1.4 Justification for the application

1.4.1 Regulatory Impact Information

A. Costs and Benefits of the application

Xylanase is an enzyme produced by submerged fermentation of *T. reesei* carrying the gene encoding the Xylanase gene from *Fusarium verticillioides*. The enzyme is characterised as an Endo-1,4-beta-xylanase (EC 3.2.1.8). A collection of information detailed in Section 3 supports the safety of the production organism and the enzyme for use in the applications outlined in Section 4.

Enzyme preparations are widely used as processing aids in the manufacture of food products. Currently no xylanase from *Fusarium verticillioides* expressed in *T. reesei* is permitted as a Processing Aid. Approval of this application would provide food processors, government and consumers with a new enzyme preparation impacting beneifts and costs as noted below.

Consumers:
There should be no quantifiable impact or costs on consumers, but there could be improved characteristics and efficiencies for starch processing and potable alcohol production.

Industry:
There are benefits to the food industry in using xylanase compared to alternatives in starch processing and potable alcohol production

In starch processing these include but are not limited to, reduced viscosity of wheat flour slurries increasing plant capacity, improved separation of starch, gluten and fibre fractions and mproved yield and purity of starch and gluten. Examples of the benefit of using xylanase in potable alcohol production include Lower mash viscosity leading to higher mash solids and lower energy consumption, effective water management from lower consumption and less water generation, and Improved sustainability from reduced GHG emissions.

Government:
There are costs or befits to government form the approval of xylanase.

B. Impact on international trade

The inclusion of Endo-1,4-beta-xylanase from *Fusarium verticillioides* expressed in *T. reesei* in the Australia New Zealand Food Standards Code as a processing aid may promote international trade on products produced with this enzyme product and reduce technical barriers to trade.

1.5 Support for the Application

Assessment Procedure No marketing or promotional activities have been undertaken for Xylanase derived from *T. reesei* containing the gene for Xylanase from *Fusarium verticillioides* in the Australia/New Zealand market. Hence at this stage, no requests from food manufacturers are provided in support of this application. However, the need and justification for use of the processing aid are discussed in Section 1.3, and it is anticipated that support from the food processing industry will be submitted during the period for public comment on the application Draft Regulatory Measure/Assessment Report.

1.6 Assessment Procedure

This application seeks to modify Schedule 18 to Standard 1.3.3 Processing Aids to permit the use of a Processing aid that is currently not permitted. Based on guidance in the Application Handbook, IFF considers General Procedure Level 1 (up to 350 hours) to be the appropriate procedure for assessment of the application.

1.7 Confidential Commercial Information (CCI)

Certain (identified) technical and manufacturing information included in Appendices B1, B3, -B6, Appendices D1-D3, Appendices E1-E5 and other information including amino acid sequences labelled with Confidential Commercial information is regarded by the applicant as **Confidential Commercial Information** and is provided in the application strictly on this basis. This information is the result of a significant research and development effort and investment by the applicant; it is not in the public domain and is considered as either proprietary or commercially sensitive. It would be disadvantageous to the applicant if this information were released into the public domain.

We have also redacted the names and contact details of all participants in the full toxicological test reports provided and discussed in Appendix B, Section 1.3.

1.8 Exclusive CommericalCapturable Benefit

According to Section 8 of the FSANZ Act, this application is not expected to confer Exclusive Capturable Commercial Benefit (ECCB).

1.9 International and other National Standard

Refer to Appendix D for further details.

1.9.1 Codex Standards

Xylanase from *Fusarium verticillioides* produced by *T. reesei* has not been reviewed by JECFA; there is no specific Codex Standard relevant to this application.

1.9.2 International legislation

Xylanase derived from *T. reesei* carrying the gene encoding the xylanase gene from *Fusarium verticillioides* has been determined to be Generally Recognized as Safe (GRAS) in the United States as a food processing aid in starch processing and potable alcohol production by a panel of scientific experts. Is also approved for various purposes in Denmark. Refer Appendix D.

1.10. Statutory declration

[redacted]

[redacted]

make the following declaration under the Oaths and Declaration Act 1959:

the information provided in this application fully sets out the matters required; and
the information is true to the best of my knowledge and belief; and
no information has been withheld which might prejudice this application to the best of my knowledge and belief.

I understand that a person who intentionally makes a false statement in a statutory declaration is guilty of an offence under section 11 of the Statutory Declarations Act 1959, and I believe that the statements in this declaration are true in every particular.

Signature _____

Declared at _____ on _____ of _____ 2022

Before me, _____

_____
Signature

1.11. Checklist

	Mandatory Requirements	Check	Page Number	Remarks
General requirements for applications	A. Form of the application	✓	N.A.	
	Table of contents	✓	1	
	Executive summary	✓	N/A	Supplied separately
	B. Applicant details	✓	2	Section 1.1
	C. Purpose of application	✓	4	Section 1.3
	D. Justification for the application	✓	4	Section 1.4
	D.1 Regulatory impact information	✓	4	Section 1.4.1
	D.1.1 Costs and benefits of the application	✓	4	Section 1.4.1
	D.1.2 Impact on international trade	✓	4	Section 1.4.1
	E Information to support the application	✓	4	Section 1.5
	E.1 Data requirements	✓	N.A.	
	F. Assessment procedure	✓	5	Section 1.6
	G. Confidential commercial information (CCI)	✓	5	Section 1.7
	H. Other confidential information	✓	5	
	I. Exclusive capturable commercial benefit (ECCB)	✓	5	Section 1.8
	J. International and other national standards	✓	5	Section 1.9
	J.1 International Standards	✓	5	Section 1.9.1
	J.2 Other national standards or regulations	✓	5	Section 1.9.2
	K. Statutory declaration	✓	6	Section 1.10
	L. Checklist	✓	7	Section 1.11
3.3.2. Processing aids	A. Technical information on the processing aid	✓	9	Section 2
	A.1 Information on the type of processing aid	✓	9	Section 2.1
	A.2 Information on the identity of the processing aid	✓	9	Section 2.2
	A.3 Information on the chemical and physical properties of the processing aid	✓	9	Section 2.3
	A.4 Manufacturing process	✓	10	Section 2.4
	A.5 Specification for identity and purity	✓	11	Section 2.5
	A.6 Analytical method for detection	✗		Not applicable for enzymes used as processing aids
	C. Information related to the safety of an enzyme processing aid	✓	13	Section 3
	C.1 General information on the use of the enzyme as a food processing aid in other countries	✓	13	Section 3.1
	C.2 Information on the potential toxicity of the enzyme processing aid	✓	14	Section 3.2
	C.3 Information on the potential allergenicity of the enzyme processing aid	✓	15	Section 3.3
	C.4 Safety assessment reports prepared by international agencies or other national government agencies, if available	✓	15	Section 3.4

	D. Additional information related to the safety of an enzyme processing aid derived from a microorganism			Section 3
	D.1 Information on the source microorganism	✓	15	Section 3.5
	D.2 Information on the pathogenicity and toxicity of the source microorganism	✓	16	Section 3.6
	D.3 Information on the genetic stability of the source organism	✓	16	Section 3.7
	E. Additional information related to the safety of an enzyme processing aid derived from a genetically-modified microorganism			Section 3
	E.1 Information on the methods used in the genetic modification of the source organism	✓	16	Section 3.8
	F Information related to the dietary exposure to the processing aid		17	Section 4
	F.1. A list of foods or food groups likely to contain the processing aid or its metabolites	✓	17	Section 4.1
	F.2 The levels of residues of the processing aid or its metabolites for each food or food group	✓	17	Section 4.2
	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	✓	17	Section 4.3
	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	✓	18	Section 4.4
	F.5 Information relating to the levels of residues in foods in other countries	✓	18	Section 4.5
	F.6 For foods where consumption has changed in recent years, information on likely current food consumption	✓	18	Section 4.6

2. Technical information

Please refer to Appendix A for further details

2.1. Type of processing aid

The Xylanase enzyme is an enzyme produced by submerged fermentation of *T. reesei*, carrying the xylanase gene from *Fusarium verticillioides*.

This Processing Aid falls into the category "Enzymes of microbial origin" from the Food Standard Code section 1.3.3-6 Enzymes.

2.2. Identity

2.2.1. Chemical/Common Name:

The systematic name of the principle enzyme activity is 4-β-D-xylan xylanohydrolase. Other names used are endo-1,4-β-xylanase, endo-(1→4)-β-xylan 4-xylanohydrolase; endo-1,4-xylanase; xylanase; β-1,4-xylanase; endo-1,4-xylanase; endo-β-1,4-xylanase; endo-1,4-β-D-xylanase; 1,4-β-xylan xylanohydrolase; β-xylanase; β-1,4-xylan xylanohydrolase; endo-1,4-β-xylanase; β-D-xylanase.

- EC number: 3.2.1.8
- CAS number: 9025-57-4

Biological source: The Xylanase enzyme is an enzyme produced by submerged fermentation of *Trichoderma reesei*, carrying the xylanase gene from *Fusarium verticillioides*.

2.2.2. Marketing Name of the processing Aid

The marketing name of this enzyme preparation will depend on the application. An example marketing name of Xylanase is Viscamyl Pro®.

2.2.3. Molecular and Structural Formula

Xylanase is a protein. This enzyme has been protein engineered. The amino acid sequence is known. Please refer to Appendix E.

2.3. Chemical and physical properties

The function of Xylanase is to catalyse the hydrolysis of the (1-4)-beta-D-xylosidic linkages that are present in the centre (endo) of xylans, including arabinoxylan.

When added during industrial wheat processing under controlled conditions, xylanase improves starch/gluten separation resulting in a reduced viscosity and improved gluten agglomeration, thus increasing their yield and purity. The benefits of the conversion of (arabino)xylans with the help of xylanase in starch processing are:

- Reduce the viscosity of wheat flour slurries increasing plant capacity. More stable and efficient processes
- Improved separation of starch, gluten and fibre fractions
- Improved yield and purity of starch and gluten
- Reduced water consumption
- Reduced lump formation and decreased maintenance of separation sieves
- Increased flexibility in the choice of raw materials

When used in potable alcohol production its used in the ethanol fermentation to reduce viscosity of the evaporated syrup by product. Using xylanase makes it possible to run the ethanol production at much higher dry solids, while keeping viscosity the same or even lower. The benefits in potable alcohol production are as follows:

- Lower mash viscosity meaning higher mash solids and lower energy consumption
- Effective water management from lower consumption and less water generation
- Higher plant capacity, higher ethanol yields
- Improved sustainability from reduced GHG emissions

Substrate specificity:
The function of Xylanase is to catalyse the hydrolysis of the (1-4)-beta-D-xylosidic linkages that are present in the centre (endo) of xylans, including arabinoxylan. The substrates for Xylanase are xylans, including arabinoxylans.

Activity:
The activity of the Xylanase is defined in NGXU. The assay is colorimetric and monitors the rate of degradation of o-nitrophenyl β-xylotrioside substrate. The release of the substrate's o-nitrophenyl is measured at 405nm on a Konelab analyser.

Temperature optimum:
The optimal temperature conditions for the activity of the food enzyme are between 42 - 69°C.

Thermal stability:
No enzyme activity is left at temperatures above 79˚C when incubating for 30 minutes.

pH optimum:
Optimal pH range lies between pH 4.8 and 7.0.

Interaction of the enzyme with different foods:
The Xylanase enzyme preparation will be used as a processing aid where the enzyme is not present or active in the final food or present in negligible amounts with no technical function in the final food.

Nutritional implication:
Xylanase is a protein and any residual amounts remaining in food consumed would accordingly have the same nutritional value. However, the use levels of Xylanase are very low, and as with other enzymes that are currently approved and used as Processing Aids, use of this preparation would not have any nutritional significance.

2.4. Manufacturing process

The enzyme is produced by a submerged fermentation process using appropriate substrate and nutrients. When fermentation is complete, the biomass is removed by centrifugation/filtration. The remaining fermentation broth containing the enzyme is filtered and concentrated. The concentrated enzyme solution is then standardised and stabilised with diluents. Finally, a polish filtration is applied.

Full details on the raw materials used for the production are provided in Appendix E. Note that this information is proprietary and "**Confidential Commercial Information**" status is requested.

The production of Xylanase is monitored and controlled by analytical and quality assurance procedures that ensure that the finished preparation complies with the specifications and is of the appropriate quality for use as a processing aid in food processing applications.

2.5. Specification for identity and purity

Impurity profile:

Appropriate GMP controls and processes are used in the manufacture of Xylanase to ensure that the finished preparation does not contain any impurities of a hazardous or toxic nature. The specification for impurities and microbial limits are as follows:

Metals:	
Lead	less than 5 mg/kg
Microbiological:	
Total viable count	less than 10,000 CFU/g
Total coliforms	less than 30 CFU/g
E. coli	absent in 25g
Salmonella	absent in 25g
Antibiotic activity	Negative by test
Production strain	Negative by test
Physical properties:	
Appearance	Off white powder

Standard for identity:

Xylanase meets the specifications laid down by the Joint FAO/WHO Expert Committee on Food Additives and the Food Chemicals Codex.

3. Safety

Refer to Appendix B for further details

3.1. Use of the enzyme as a food processing aid in other countries

Enzyme products are developed for a specific function, i.e. to catalyse a specific chemical reaction. That reaction determines the IUBMB classification. Enzyme variants may be selected to have a better performance of that function under the specific conditions of the application (e.g. temperature or pH). Enzymes of a certain IUBMB classification share conserved structural elements, called domains, which are needed for their specific function. As such the enzymes of our approval procedures do resemble those already permitted by FSANZ both in function and in structure.

Figure 1 below shows an example of natural variation of alpha-amylases. The same holds for any other enzyme type. While significant differences in sequence amongst the various species exist, they all catalyse the same reaction and therefore fit under the same IUBMB entry. There will also be natural variation within one species. All this also applies to the enzymes under the current approval procedures by FSANZ:

% amino acid sequence identity	*B. amyloliquefaciens*	*B. licheniformis*	*G. stearothermophilus*	*A. niger*	*A. oryzae*	*Z. mays*	*O. sativa*	*H. vulgare*	*P. vulgaris*	*H. sapiens*
Bacillus amyloliquefaciens	100									
Bacillus licheniformis	80	100								
Geobacillus stearothermophilus	65	65	100							
Aspergillus niger	21	21	22	100						
Aspergillus oryzae	23	24	24	66	100					
Zea mays (corn)	24	26	25	28	27	100				
Oryza sativa (rice)	25	27	25	27	26	89	100			
Hordeum vulgare (barley)	25	23	24	25	28	70	69	100		
Phaseolus vulgaris (bean)	26	27	25	24	27	67	65	64	100	
Homo sapiens (human)	25	33	29	22	28	23	22	23	24	100

α-amylases in nature have divergent

amino acid sequences but have the same catalytic activity and IUBMB number

Figure 1. Variation of enzymes in nature.

The expressed mature enzyme amino acid sequence of Xylanase shows a clear conserved Glyco_hydro_11 superfamily sequence domain, characteristic for endo-1,4-beta-xylanase activities.

Xylanase enzyme, the subject of this dossier, is one of the approved endo-1,4-xylanase enzymes on Schedule 18 of the ANZ Food Standards Code. In our case the enzyme protein is expressed from *Trichoderma reesei*. The identity between the FSANZ approved endo-1,4-xylanase (*A. niger, A.oryzae, A. aculeatus, T. lanuginosus, B. amyloliquefaciens, B. subtilis, B. licheniformis, H. insolens, T. reesei*) ranges from 16.9 - 75.5%. It is good to realise that the endo-1,4-xylanase sequences within one species can show strain dependent amino acid sequence variability. Also, several microorganism species contain more than one endo-1,4-xylanase encoding genes with different sequences (e.g. *H. insolens* XynA and XynB).

Xylanase enzyme derived from *T. reesei*, carrying the xylanase gene from *Fusarium verticillioides* has been determined to be GRAS in the United States, and been used for potable alcohol production and starch processing in other countries such as Denmark since 2018. There have not been any adverse events reported since Xylanase has been in commercial use in these countries.

Please refer to section 1.9 and Appendix D for details on the different approval procedures in the countries listed above.

3.2. Toxicity of the enzyme

Toxin homology study
A BLAST search for homology of the xylanase sequence against the complete Uniprot database (http://www.uniprot.org/), was performed, with a threshold E-value of 0.1. The majority of matches were endo-1,4-beta xylanases, with none of the top 250 database matches being annotated as either toxin or venom.

In addition, a specific BLAST search for homology of the mature xylanase sequence was performed against the Uniprot animal toxin database. This yielded no matches.

Therefore, the xylanase sequence does not share homology with a known toxin or venom sequence.

Safe Strain Lineage concept
The Safe Strain Lineage concept has been discussed by Pariza and Johnson (2001) in their publication on the safety of food enzymes and is commonly utilised by enzyme companies in the determination of the safety of their products for specific uses, as appropriate.

The primary issue in evaluating the safety of a production strain is its toxigenic potential, specifically the possible synthesis by the production strain of toxins that are active via the oral route. The toxigenic potential of the production organism is confined to the Total Organic Solid (TOS) originating from the fermentation.

As the toxicological evaluation is based on the TOS originating from fermentation of the production organism, studies conducted on strains from the Safe Strain Lineage can support other production strains pertaining to this same Safe Strain Lineage.

Although *T. reesei* is scientifically determined by IFF as a Safe Strain Lineage, the food enzyme object of the current dossier is supported by toxicological studies on the specific food enzyme object of this dossier. The toxicological studies on *T. reesei* LVS-ETD-FVEXYN4-CL8-D3#15.2.3 are thus one of the pillars supporting the IFF *T. reesei* Safe Strain Lineage. The position of the food enzyme in the IFF *Trichoderma reesei* Safe Strain Lineage is presented in Appendix B2.

Toxicological testing
To assess the safety of Xylanase, different endpoints of toxicity were investigated and are evaluated and assessed in this document:

- Ames test: no mutagenic activity under the given test conditions
- Chromosomal aberrations: no clastogenic activity under the given test conditions
- 90-day oral toxicity on rats: the NOAEL (no observed adverse effect level) is established at the highest dose tested, 1000 mg (TOS)/kg bw/day equivalent to 967.8 mg total protein/kg bw/day in male and female rats.

A summary of the results of the studies can be found in Appendix B.

In addition, safety was further assessed according to the decision tree in the Pariza-Johnson guidelines (2001) for assuring the safety of a new enzyme preparation

3.3. Allergenicity of the enzyme

Bioinformatic analyses based on sequence homology determined that the *Fusarium verticillioides* xylanase is unlikely to pose a risk of food allergenicity. Refer to Appendix B for additional information on the safety of the enzyme as to its allergenicity potential.

An allergen statement is given in Appendix A9. Wheat derived glucose is used in fermentation, this material is exempt from food allergen labelling according S9-3, Schedule 9 of the Joint Australia New Zealand Food Standards Code

3.4. Safety assessment reports prepared by international agencies or other national government agenices, if available

As discussed in section 1.9, Xylanase from *Fusarium verticillioides* produced by *T. reesei* has been reviewed determined to be GRAS in the United States, and approved in and Denmark for various purposes. Refer Appendix D for safety reports/approval letters.

3.5. Information on the source organism.

The production organism strain LVS-ETD-FVEXYN4-CL8-D3#15.2.3 is a strain of *T. reesei* which has been genetically modified by IFF to overexpress a xylanase gene from *Fusarium verticillioides*.

T. reesei has a long history of safe use in industrial scale enzyme production. The safety of this species as an industrial enzyme producer has been reviewed by Nevalainen et al. (1994), Blumenthal (2004) and Olempska-Beer et al. (2006). The organism is considered non-pathogenic for humans and does not produce fungal toxins or antibiotics under conditions used for enzyme production. It is generally recognised as a safe production organism and is the source organism of a range of enzyme preparations that are used as processing aids in the international food and feed industries. It is also considered as suitable for Good Industrial Large Scale Practice (GILSP) worldwide and meets the criteria for a safe production microorganism as described by Pariza and Johnson (2001). The xylanase gene was placed under the expression signals of the endogenous *T. reesei* cbh1 gene promoter and terminator linked to the endogenous *T. reesei* orotate phosphoribosyl transferase (pyr2) gene as a selectable marker. Insertion of the fragment into the T. reesei genome was by non-homologous recombination at unpredictable site(s) and in unpredictable copy number.

Full details of the gene and recombinant microorganism are provided in Appendix E. Note that this information is proprietary and "Confidential Commercial Information" status is requested.

3.6. Pathogenicty and toxicity of the source micro-organism

Trichoderma reesei was first isolated from nature in 1944. The original isolate, QM6a (Mandels and Reese, 1957), and its subsequent derivatives have been the subject of intense research due to their usefulness in the production of cellulases.

A literature search was conducted on August 28, 2017 using the searching term "*Trichoderma reesei*" and "food safety OR toxin OR toxicology OR pathogen" on PubMED resulting in 43 records. A review of the literature search uncovered no reports that implicate *Trichoderma reesei* in any way with a disease situation, intoxication, or allergenicity among healthy adult human and animals.

Strain QM6a and its derivatives have been safe producers of commercial cellulase enzyme preparations for food applications. The industrial enzyme preparations are still confirmed by the enzyme manufacturers not to have antibiotic activity according to the specifications recommended by JECFA (2006).

T. reesei has a long history of safe use in industrial scale enzyme production. The safety of this species as an industrial enzyme producer has been reviewed by Nevalainen *et al*. (1994) and Blumenthal (2004). The organism is considered non-pathogenic for humans and does not produce fungal toxins or antibiotics under conditions used for enzyme production. It is generally considered a safe production organism and is the source organism of a range of enzyme preparations that are used as processing aids in the international food and feed industries. It is listed as a safe production organism for cellulases by Pariza and Johnson (2001) and Olempska-Beer *et al.* (2006), and various strains have been approved for the manufacture of commercial enzyme preparations by Food Standards Australia New Zealand, and internationally, for example, in Canada (Food and Drugs Act Division 16, Table V), the United States (21CFR § 184.1250), Mexico, Brazil, France, Denmark, China, and Japan. Further details are discussed in Appendix B

3.7. Genetic stability of the source organism

The parental strain of the production strain *Trichoderma reesei* QM6a and its derivatives have been used for industry scale enzyme manufacturing for decades by IFF and its parental companies and has demonstrated stable enzyme expression even at large scale fermentation. Please also refer to Appendix B2 for list of example enzyme preparations produced using QM6a and its derivatives. Furthermore, the production strain has demonstrated to be 100% stable as confirmed by genome sequencing. Refer also section 3.6

3.8. Method used in the genetic modifiction of the source organism

The production organism of the Xylanase preparation, the subject of this submission, is *T. reesei* strain LVS-ETD-FVEXYN4-CL8-D3#15.2.3. It is derived by recombinant DNA methods from strain RL-P37. The purpose of this genetic modification is to enhance xylanase production levels. RL-P37, a commercial production strain, is derived, as a result of several classical mutagenesis steps, from the well-known wild-type strain QM6a. Virtually all strains used all over the world for industrial cellulase production today are derived from QM6a. The donor organism is *Fusarium verticillioides*. Xylanase expression cassette was integrated into the host genome. Full details of the genetic modifications are provided in Appendix E2 (Confidential Commercial Information).

The genetic stability of the inserted gene has been demonstrated by genome sequencing. NGS sequence analysis was used to characterise the production strain for the insertion site prior to and at the end of a 220hr fermentation protocol. The production strain is completely stable after industrial scale fermentation, judged by xylanase production derived from the integrated expression cassettes and comparative genomic analysis of the strain at the beginning and end of fermentation.

Full details of the genetic modifications and stability of the inserted genes are provided in Appendix E1-E3. Note that this information is proprietary and "**Confidential Commercial Information**" status is requested.

4. Dietary exposure

Refer to Appendix C for further details

4.1. List of food or food groups likely to contain the enzyme or its metabolites

According to the food group classification system used in Standard 1.3.1-Food Additives Schedule 15 (15-5), Xylanase will be used in:

- 11.2 Sugars and sugar syrups
- 14.2. Alcoholic beverages (including alcoholic beverages that have had the alcohol reduced or removed)

4.2. Levels of residues in food

The proposed application rate of Xylanase in its intended application is listed below.

Application	Raw material (RM)	Recommended use levels (mg TOS/kg RM)	Maximal recommended use levels (mg TOS/kg RM)
Starch processing	Wheat	1.71-4.28	4.28
Starch processing	Starch	1.07-4.28	4.28

IFF expects the Xylanase to be inactivated or removed during the subsequent production and refining processes for all applications.

In potable alcohol production and starch processing, Xylanase performs its technological function to contribute to an improved and consistent process. When producing potable alcohol, the enzyme is used during slurry mixing. In starch processing xylanase is added to the mixing step of wheat flour and water.

The most appropriate way to estimate the human consumption in the case of food enzymes is using the Budget Method (Hansen, 1966; Douglass *et al.*, 1997). This method enables one to calculate a Theoretical Maximum Daily Intake (TMDI) based on conservative assumptions regarding physiological requirements for energy from food and the energy density of food rather than on food consumption survey data. The Budget Method was originally developed for determining food additive use limits and is known to result in conservative estimations of the daily intake.

Based on the raw materials used in the various food processes, the recommended use levels of the enzyme Xylanase, for the calculation of the TMDI, the maximum use levels are chosen. The TMDI is calculated on basis of the maximal values found in food and beverages multiplied by the average consumption of food and beverages per kg body weight/day. Consequently, the TMDI will be: 0.011 mg TOS/kg body weight/day. The NOAEL has been determined for Xylanase to be at 1000 mg total protein/kg bw/day (equivalent to 967.8 mg TOS/kg bw/day). Based on a worst-case scenario of daily food consumption, the NOAEL would offer a 90,909-fold margin of safety. It should be stressed that this Total TMDI is based on conservative assumptions and represents a highly exaggerated value. Please refer to Appendix C for details.

4.3. Likely level of consumption of foods or food groups not currently listed in the most recent Australian or New Zealand National Nutrition Surveys (NNSs)

Not applicable. Xylanase is not expected to be used in production of any foods or food groups that are currently not listed in NNSs. If such usage arises, an application would be made to inform FSANZ.

4.4. 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

The enzyme would be used as a processing aid in about:

- 20% of the tonnage of potable alcohol products sold in Australia and New Zealand
- 20% of sweetener sold in Australia and New Zealand

4.5. Levels of residues in food in other countries

Applications and levels of use of the Xylanase preparation in other countries is the same as presented in section 4.2.

4.6. Likely current food consumption for foods where consumption has changed in recent years

Not applicable. Consumption of foods (alcoholic drinks and sugar syrup/sweeteners) produced with Xylanase is not expected to have a significant change.

5. References

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