



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

7-05

5 October 2005

## **INITIAL ASSESSMENT REPORT**

### **APPLICATION A569**

### **LIPASE FROM *HANSENULA POLYMORPHA* AS A PROCESSING AID (ENZYME)**

**DEADLINE FOR PUBLIC SUBMISSIONS: 6pm (Canberra time) 16 November 2005**

**SUBMISSIONS RECEIVED AFTER THIS DEADLINE**

**WILL NOT BE CONSIDERED**

*(See 'Invitation for Public Submissions' for details)*

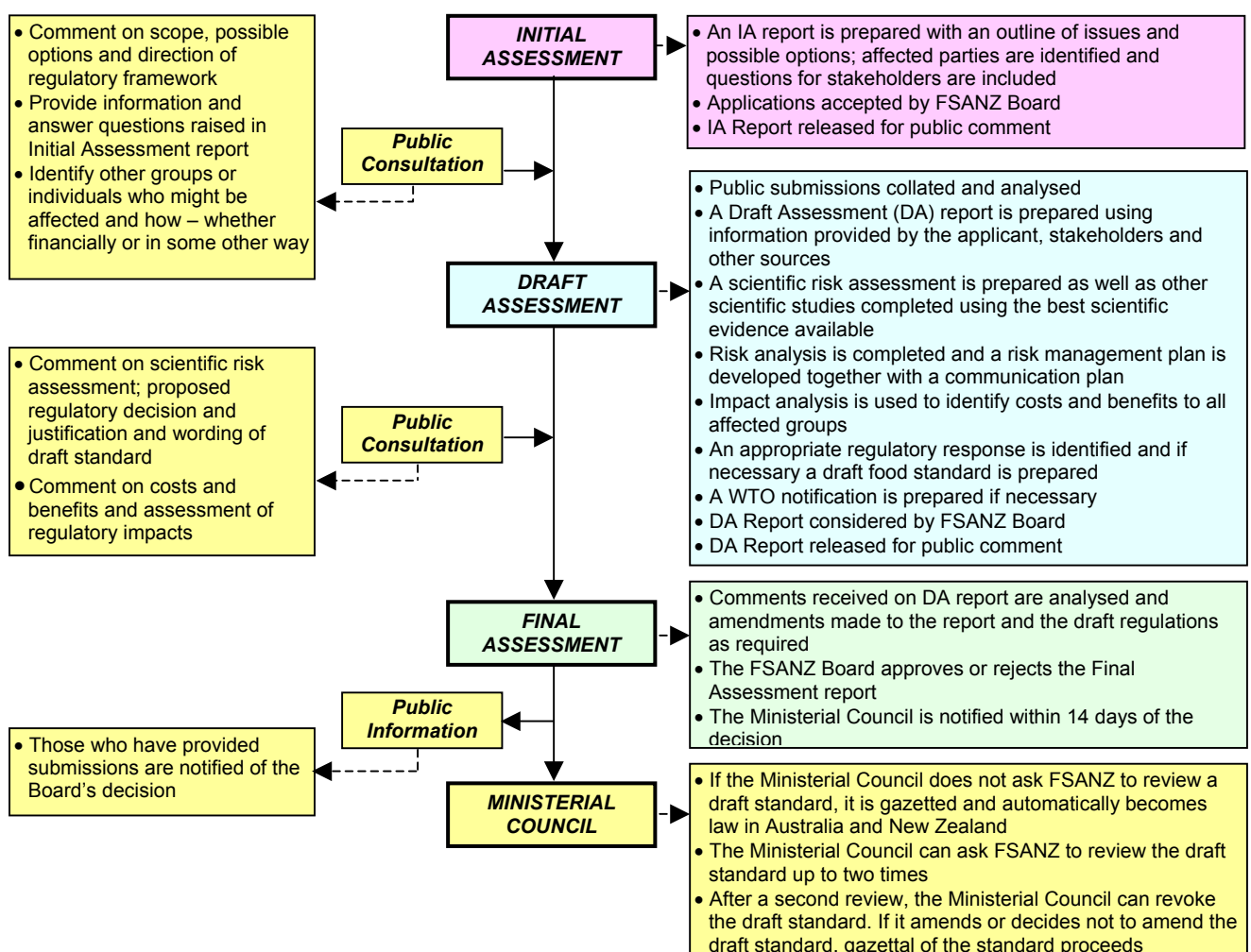
## FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

FSANZ's role is to protect the health and safety of people in Australia and New Zealand through the maintenance of a safe food supply. FSANZ is a partnership between ten Governments: the Australian Government; Australian States and Territories; and New Zealand. It is a statutory authority under Commonwealth law and is an independent, expert body.

FSANZ is responsible for developing, varying and reviewing standards and for developing codes of conduct with industry for food available in Australia and New Zealand covering labelling, composition and contaminants. In Australia, FSANZ also develops food standards for food safety, maximum residue limits, primary production and processing and a range of other functions including the coordination of national food surveillance and recall systems, conducting research and assessing policies about imported food.

The FSANZ Board approves new standards or variations to food standards in accordance with policy guidelines set by the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council) made up of Australian Government, State and Territory and New Zealand Health Ministers as lead Ministers, with representation from other portfolios. Approved standards are then notified to the Ministerial Council. The Ministerial Council may then request that FSANZ review a proposed or existing standard. If the Ministerial Council does not request that FSANZ review the draft standard, or amends a draft standard, the standard is adopted by reference under the food laws of the Australian Government, States, Territories and New Zealand. The Ministerial Council can, independently of a notification from FSANZ, request that FSANZ review a standard.

The process for amending the *Australia New Zealand Food Standards Code* is prescribed in the *Food Standards Australia New Zealand Act 1991* (FSANZ Act). The diagram below represents the different stages in the process including when periods of public consultation occur. This process varies for matters that are urgent or minor in significance or complexity.



## INVITATION FOR PUBLIC SUBMISSIONS

FSANZ has prepared an Initial Assessment Report of Application A569, which includes the identification and discussion of the key issues.

FSANZ invites public comment on this Initial Assessment Report for the purpose of preparing an amendment to the Code for approval by the FSANZ Board.

Written submissions are invited from interested individuals and organisations to assist FSANZ in preparing the Draft Assessment for this Application. Submissions should, where possible, address the objectives of FSANZ as set out in section 10 of the FSANZ Act. Information providing details of potential costs and benefits of the proposed change to the Code from stakeholders is highly desirable. Claims made in submissions should be supported wherever possible by referencing or including relevant studies, research findings, trials, surveys etc. Technical information should be in sufficient detail to allow independent scientific assessment.

The processes of FSANZ are open to public scrutiny, and any submissions received will ordinarily be placed on the public register of FSANZ and made available for inspection. If you wish any information contained in a submission to remain confidential to FSANZ, you should clearly identify the sensitive information and provide justification for treating it as commercial-in-confidence. Section 39 of the FSANZ Act requires FSANZ to treat in-confidence, trade secrets relating to food and any other information relating to food, the commercial value of which would be, or could reasonably be expected to be, destroyed or diminished by disclosure.

Submissions must be made in writing and should clearly be marked with the word 'Submission' and quote the correct project number and name. Submissions may be sent to one of the following addresses:

**Food Standards Australia New Zealand**  
**PO Box 7186**  
**Canberra BC ACT 2610**  
**AUSTRALIA**  
**Tel (02) 6271 2222**  
**[www.foodstandards.gov.au](http://www.foodstandards.gov.au)**

**Food Standards Australia New Zealand**  
**PO Box 10559**  
**The Terrace WELLINGTON 6036**  
**NEW ZEALAND**  
**Tel (04) 473 9942**  
**[www.foodstandards.govt.nz](http://www.foodstandards.govt.nz)**

**Submissions need to be received by FSANZ by 6pm (Canberra time) 16 November 2005.**

Submissions received after this date will not be considered, unless agreement for an extension has been given prior to this closing date. Agreement to an extension of time will only be given if extraordinary circumstances warrant an extension to the submission period. Any agreed extension will be notified on the FSANZ Website and will apply to all submitters.

While FSANZ accepts submissions in hard copy to our offices, it is more convenient and quicker to receive submissions electronically through the FSANZ website using the Standards Development tab and then through Documents for Public Comment. Questions relating to making submissions or the application process can be directed to the Standards Management Officer at the above address or by emailing [slo@foodstandards.gov.au](mailto:slo@foodstandards.gov.au).

Assessment reports are available for viewing and downloading from the FSANZ website. Alternatively, requests for paper copies of reports or other general inquiries can be directed to FSANZ's Information Officer at either of the above addresses or by emailing [info@foodstandards.gov.au](mailto:info@foodstandards.gov.au).

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## Executive Summary

FSANZ received an Application on 29 July 2005 from Danisco Australia Pty Ltd (submitted by Axiome Pty Ltd), to amend Standard 1.3.3 – Processing Aids of the Code to approve an enzyme, lipase, triacylglycerol (EC number [3.1.1.3]), as a processing aid. Lipase, triacylglycerol is produced, using recombinant DNA techniques, from the host yeast *Hansenula polymorpha* containing the gene coding for lipase, triacylglycerol from the mould *Fusarium heterosporum*.

This Initial Assessment Report is not a detailed assessment of the Application but rather an assessment of whether the Application should undergo further consideration. The Report is based mainly on information provided by the Applicant and has been written to assist in identifying the affected parties and to outline expected relevant issues to complete the assessment. The information needed to complete the assessment will include responses received from public submissions.

Processing aids are required to undergo a pre-market safety assessment before approval for use in Australia and New Zealand. There is currently approval for the use of different microbial and animal sources of lipase, triacylglycerol in the Code. As well there are other applications which are currently being assessed for alternative microbial sources of this enzyme, primarily for cheese flavour manufacture.

The objective of the assessment is to determine whether the Code should be amended to permit the use of lipase, triacylglycerol, from the host yeast *H. polymorpha* containing the gene coding for lipase, triacylglycerol isolated from the mould *F. heterosporum*. An assessment of the nomenclature of both *H. polymorpha* and *F. heterosporum* will be made at Draft Assessment.

The host organism, the yeast *H. polymorpha* is an approved host for another genetically modified source organism for the enzyme hexose oxidase which is listed in the Code. The Applicant claims the genetic modifications are well characterised and specific, utilising well-known plasmids so that the genetically modified *H. polymorpha* is considered a safe source organism for the enzyme.

The Applicant claims lipase, triacylglycerol catalyses the hydrolysis of triglycerides, as well as phospholipids and galactolipids. It is claimed that the major application utilising the enzyme is in bread making, to improve dough stability and dough handling properties, and to improve the bread volume and crumb homogeneity. The Applicant also claims the enzyme can be used to treat egg yolk destined for baking or for the production of mayonnaise and salad dressing, and for the degumming of edible oils.

The enzyme preparation meets the international specifications for enzymes, namely the Food Chemicals Codex (5<sup>th</sup> Edition, 2004) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA), Compendium of Food Additive Specifications, FAO Food and Nutrition Paper 52, Volume 1, Annex 1, Addendum 9, 2001 (General Specifications and Considerations for Enzyme Preparations Used in Food Processing).

A self-affirmed GRAS determination has been made under the US requirements of the Code of Federal Regulations. An expert panel evaluated the safety of using the enzyme obtained from this source and have concluded that it is Generally Recognized As Safe (GRAS). The summary report (27 May 2005) of this evaluation is provided in the Application.

## **FSANZ DECISION**

**FSANZ accepts this Application seeking approval for the enzyme lipase, triacylglycerol from *H. polymorpha* containing the gene coding for lipase, triacylglycerol from *F. heterosporum*. Public submissions are now being sought.**

The Application has been assessed against the requirements of section 13 of the FSANZ Act and accepted for the following reasons:

- The Application seeks approval for a new enzyme from a microbial source as a processing aid.
- Microbial enzymes and their sources are listed in the Table to clause 17 of Standard 1.3.3 of the Code. There is currently no approval for lipase, triacylglycerol from the source *H. polymorpha*, containing the gene for lipase, triacylglycerol isolated from *F. heterosporum* in this Table.
- The Application relates to a matter that warrants a variation to Standard 1.3.3, if further assessment supports such a variation.
- This Application is not so similar to any previous application that it ought not be accepted.
- There are no other regulatory measures, than a variation to the Code available to permit the use of this processing aid.
- There is nothing to indicate, at this stage, that the costs, that would arise from a variation to the Code approving the new enzyme, outweigh the direct and indirect benefits to the community, Government or industry of the approval.

The Application is recommended for further consideration, so FSANZ now seeks public submissions to assist in assessing the Application.

## 1. Introduction

FSANZ received an Application on 29 July 2005 from Danisco Australia Pty Ltd (submitted by Axiome Pty Ltd), to amend Standard 1.3.3 – Processing Aids of the Code to approve an enzyme, lipase, triacylglycerol (EC number [3.1.1.3]), as a processing aid. Lipase, triacylglycerol is produced, using recombinant DNA techniques, from the host yeast *H. polymorpha* containing the gene coding for lipase, triacylglycerol from the mould *F. heterosporum*.

The Applicant claims lipase, triacylglycerol catalyses the hydrolysis of triglycerides, as well as phospholipids and galactolipids. It is claimed that the major application utilising the enzyme is in bread making, to improve dough stability and dough handling properties, and to improve the bread volume and crumb homogeneity. The Applicant also claims the enzyme can be used to treat egg yolk destined for baking or for the production of mayonnaise and salad dressing, and for the degumming of edible oils.

## 2. Regulatory Problem

Processing aids are required to undergo a pre-market safety assessment before approval for use. A processing aid is a substance used in the processing of raw materials, foods or ingredients, to fulfil a technological purpose relating to treatment or processing, but does not perform a technological function in the final food.

The Table to clause 17 of Standard 1.3.3 contains a list of permitted enzymes of microbial origin. There are a number of approved sources of the enzyme, lipase, triacylglycerol, but not the source *H. polymorpha* containing the gene coding for lipase, triacylglycerol from the mould *F. heterosporum*. The yeast *H. polymorpha* is an approved host for a genetically modified (GM) source organism of the hexose oxidase enzyme, being the source *H. polymorpha*, containing the gene for hexose oxidase isolated from *Chondrus crispus*.

## 3. Objective

The objective of this assessment is to determine whether it is appropriate to amend the Code to permit the use of lipase, triacylglycerol from *H. polymorpha* containing the gene coding for lipase, triacylglycerol from *F. heterosporum*.

In developing or varying a food standard, FSANZ is required by its legislation to meet three primary objectives which are set out in section 10 of the FSANZ Act. These are:

- the protection of public health and safety;
- the provision of adequate information relating to food to enable consumers to make informed choices; and
- the prevention of misleading or deceptive conduct.

In developing and varying standards, FSANZ must also have regard to:



- the need for standards to be based on risk analysis using the best available scientific evidence;
- the promotion of consistency between domestic and international food standards;
- the desirability of an efficient and internationally competitive food industry;
- the promotion of fair trading in food; and
- any written policy guidelines formulated by the Ministerial Council.

## **4. Background**

### **4.1 Historical Background**

Lipases have a large number of uses both in the food industry as well as in the broader biotechnology area. In the biotechnology field, lipases can act as versatile biocatalysts that can perform hydrolysis, interesterification, esterification, alcoholysis, acidolysis and aminolysis<sup>1</sup>.

In the food industry, lipases have a number of uses, which have increased in the last few years. They can be used in the fruit juice industry, baked goods, vegetable fermentation and dairy industries. Lipases have traditionally been used in the oils and fats industries where lipases catalyse the cleavage of fatty acids from triglycerides in fats. Lipases can be used for de-gumming purposes in the fats and oils industries. They can also be used to improve the emulsifying properties of ingredients (such as lecithin and egg yolk) during food processing.

Lipase, triacylglycerol (EC number [3.1.1.3]) is currently approved as an enzyme with different microbial sources in the Table to clause 17 – Permitted enzymes of microbial origin of Standard 1.3.3. It is also listed in the Table to clause 15 – Permitted enzymes of animal origin, as Lipase (EC [3.1.1.3]), being sourced from bovine stomach; salivary glands or forestomach of calf, kid or lamb; porcine or bovine pancreas.

There is another different lipase listed in Table to clause 17 of Standard 1.3.3, called lipase, monoacylglycerol with EC number of [3.1.1.23].

### **4.2 Work Plan Classification**

This Application had been provisionally rated as Category of Assessment 2 (level of complexity) and placed in Group 3 on the FSANZ standards development Work Plan. This Initial Assessment confirms these ratings. Further details about the Work Plan and its classification system are given in *Information for Applicants* at [www.foodstandards.gov.au](http://www.foodstandards.gov.au).

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<sup>1</sup> Pandey, A.; Benjamin, S.; Soccol, C.R.; Nigam, P.; Krieger, N. and Soccol, V.T. (1999) The realm of microbial lipases in biotechnology, *Biotechnol. Appl. Biochem.* **29**, 119-131.

## 5. Relevant Issues

### 5.1 Nature and technological justification of the enzyme

In the Table to clause 17 – Permitted enzymes of microbial origin of Standard 1.3.3 of the Code the name of this enzyme of this Application is lipase, triacylglycerol.

The systematic name of the enzyme is triacylglycerol acylhydrolase, while the common name is triacylglycerol lipase<sup>2</sup>. Other names include lipase, triglyceride lipase and tributyrase.

It has the Enzyme Commission (EC) number of [3.1.1.3] and a Chemical Abstracts Service (CAS) number of 9001-62-1.

The enzyme preparation is an off-white to brownish coloured powder which is freely soluble in water. The enzyme is stable between pH 5 and 7 with optimum pH stability at 6.5. The enzyme activity occurs between pH 4 to 10, with its optimum activity at pH 8. The optimum temperature of use is approximately 40°C. It is not thermally stable above 45°C in an aqueous solution. The molecular weight of the enzyme was determined to be 30 kDa by the SDS-PAGE gel method.

Lipases are enzymes that catalyse the cleavage of triglycerides to fatty acids. The enzyme is characterised by its ability to catalyse the reaction:



The lipase, triacylglycerol enzyme preparation is produced by submerged fermentation using a selected strain of the yeast *H. polymorpha* that has the gene coding for lipase, triacylglycerol isolated from *F. heterosporum* inserted by recombinant DNA techniques. After fermentation is completed the biomass is removed by centrifugation and filtration. The supernatant fermentation broth which contains the enzyme is filtered and then concentrated by ultra-filtration. The ultra-filtrate is then sterile filtered and finally spray dried or granulated onto a food grade carrier such as wheat starch. The manufacturing process is that commonly used to produce enzymes from microbial sources.

It is unlikely that there are any dietary or nutrition implications with this Application. The enzyme is to be used as a processing aid and the majority of the enzyme will be removed from the final product as part of the manufacturing process. Some small proportion of the enzyme may remain in the final products but it will have been inactivated to a protein, having the same nutritional value as protein. The enzyme will be used at very low levels. Enzymes and their reaction by-products, diacylglycerol and fatty acids, are natural components of food and no different to other constituents of food.

The technological justification will be investigated more fully in a Food Technology Report, as part of the Draft Assessment Report.

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<sup>2</sup> International Union of Biochemistry and Molecular Biology (IUBMB) Enzyme Nomenclature <http://www.chem.qmul.ac.uk/iubmb/enzyme/EC3/1/1/32.html>, accessed on 5 May 2005

## 5.2 Safety assessment

The host microorganism, the yeast *H. polymorpha*, is a host for another genetically modified source organism of an approved enzyme, hexose oxidase, in the Table to clause 17 of Standard 1.3.3. The Applicant states that recombinant strains of *H. polymorpha* are used for commercial production of the hepatitis B vaccine and a number of other medical vaccines. The Applicant quotes literature which describes *H. polymorpha* as a safe production organism, which does not contain pyrogens, pathogens or 'viral inclusions'.

The mould *F. venenatum* has not been used as a donor organism for any approved enzyme sources in the Table to clause 17 of Standard 1.3.3 or within the Code. *F. venenatum* also does not have a long history of safe use in food. The Applicant has performed literature searches to indicate the safety of the donor, which will be assessed as part of the Safety Assessment Report.

The Applicant believes the genetic modifications to produce the enzyme source are well characterised and specific, utilising well-known plasmids for the vector constructs, and because the introduced genetic material does not encode and express any known harmful or toxic substances, it is considered a safe source. The Applicant states that when the safety is assessed according to the decision tree in the paper by Pariza-Johnson on the safety of a new enzyme preparations<sup>3</sup>, the enzyme preparation is 'accepted'. The workings of the decision tree are provided in the Application.

The Applicant has provided the following studies:

- Acute oral toxicity studies in rats.
- 13 weeks sub-chronic oral toxicity study in rats.
- Test for mutagenic activity (Ames test).
- *In vitro* chromosomal aberration study.

These studies as well as the safety of the genetic modifications will be assessed as part of the Safety Assessment Report prepared for the Draft Assessment report.

A microbiological assessment of the nomenclature of the source organism, *H. polymorpha* containing the gene coding for lipase, triacylglycerol from *F. heterosporum* will be made during the Draft Assessment. That is, both the names *H. polymorpha* and *F. heterosporum* will be assessed as whether they are appropriate.

## 5.3 Other international regulatory standards

The same enzyme from the same Applicant has recently (27 May 2005) been deemed self-affirmed GRAS in the USA (the summary report of the GRAS Expert panel is contained in the Application).

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<sup>3</sup> Pariza, M.W. and Johnson, E.A. (2001) Evaluating the safety of microbial enzyme preparations used in food processing: update for a new century, *Regulatory Toxicology and Pharmacology*, **33**:173-186.

The Applicant claims the enzyme preparation complies with the specifications for enzyme preparations in the Food Chemicals Codex, 5<sup>th</sup> Edition, 2004<sup>4</sup> and JECFA Compendium of Food Additive Specifications, Volume 1, Annex 1, Addendum 9 2001, (General Specifications and Considerations for Enzyme Preparations Used in Food Processing)<sup>5</sup>.

## 6. Regulatory Options

FSANZ is required to consider the impact of various regulatory (and non-regulatory) options on all sectors of the community, which includes consumers, food industries and governments in Australia and New Zealand. The benefits and costs associated with the proposed amendment to the Code will be analysed using regulatory impact principles at Draft Assessment.

There are no options other than a variation to the Code for this Application. Therefore the two regulatory options available for this Application are:

**Option 1.** Not approve the use of lipase, triacylglycerol from *H. polymorpha* containing the gene coding for lipase, triacylglycerol from *F. heterosporum* as a processing aid.

**Option 2.** Approve lipase, triacylglycerol from *H. polymorpha* containing the gene coding for lipase, triacylglycerol from *F. heterosporum* as a processing aid.

## 7. Impact Analysis

### 7.1 Affected Parties

The affected parties to this Application include the following:

1. those sectors of the food industry wishing to produce and market food products manufactured using this enzyme;
2. consumers; and
3. Australian, State, Territory and New Zealand Government agencies that enforce food regulations.

### 7.2 Impact Analysis

In the course of developing food regulatory measures suitable for adoption in Australia and New Zealand, FSANZ is required to consider the impact of all options on all sectors of the community, including consumers, the food industry and governments. The regulatory impact assessment identifies and evaluates, though is not limited to, the costs and benefits of the regulation, and its health, economic and social impacts.

The regulatory impact of the proposed change will be assessed at Draft Assessment.

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<sup>4</sup> Food Chemicals Codex (2004), National Academy of Sciences, Food and Nutrition Board, Committee on Food Chemical Codex, 5<sup>th</sup> Edition, National Academy Press, Washington DC, pp 146-152.

<sup>5</sup> Joint FAO/WHO Expert Committee on Food Additives (JECFA) (2001). General specifications and considerations for enzyme preparations used in food processing. FAO Food and Nutrition Paper 52, Addendum 9, pp 37-39.

## **8. Consultation**

### **8.1 Public consultation**

The Initial Assessment Report is not a detailed assessment of this Application but rather an assessment of whether the Application should undergo further consideration. FSANZ is seeking public comment in order to assist in assessing this Application at Draft Assessment. A further round of public comment will occur after the Draft Assessment Report is completed to assist in the Final Assessment.

FSANZ is seeking public comment to assist in assessing the Application. Comments on, but not limited to, the following would be useful:

- technological justification for the use of the enzyme;
- safety considerations of using the enzyme and the source organism;
- appropriate nomenclature of the source organism both the donor organism, *F. heterosporum* and the host, *H. polymorpha*;
- other scientific aspects; and
- various costs and benefits of its use, including how various food industries anticipate they may use the enzyme and in which foods, to assist FSANZ in assessing the impact of approving the enzyme.

### **8.2 World Trade Organization (WTO)**

As members of the World Trade Organization (WTO), Australia and New Zealand are obligated to notify WTO member nations where proposed mandatory regulatory measures are inconsistent with any existing or imminent international standards and the proposed measure may have a significant effect on trade.

Amending the Code to approve the enzyme lipase, triacylglycerol from *H. polymorpha* containing the gene coding for lipase, triacylglycerol from *F. heterosporum* as a processing aid is unlikely to have a significant effect on trade. The enzyme preparation is consistent with the international specifications for food enzymes of Food Chemicals Codex (5<sup>th</sup> Edition, 2004) and JECFA so there does not appear to be a need to notify the WTO. This issue will be fully considered at Draft Assessment and, if necessary, notification will be recommended to the agencies responsible in accordance with Australia's and New Zealand's obligations under the WTO Technical Barrier to Trade (TBT) or Sanitary and Phytosanitary Measure (SPS) Agreements. This will enable other WTO member countries to comment on proposed changes to standards where they may have a significant impact on them.

## **9. Conclusion**

FSANZ accepts this Application seeking approval for the enzyme lipase, triacylglycerol from *H. polymorpha* containing the gene coding for lipase, triacylglycerol from *F. heterosporum*. Public submissions are now being sought.

The Application has been assessed against the requirements of section 13 of the FSANZ Act and accepted for the following reasons:

- The Application seeks approval for a new enzyme from a microbial source as a processing aid.
- Microbial enzymes and their sources are listed in the Table to clause 17 of Standard 1.3.3 of the Code. There is currently no approval lipase, triacylglycerol from *H. polymorpha* containing the gene coding for lipase, triacylglycerol from *F. heterosporum* in this Table.
- The Application relates to a matter that warrants a variation to Standard 1.3.3, if further assessment supports such a variation.
- This Application is not so similar to any previous application that it ought not be accepted.
- There are no other measures, than a variation to the Code available to permit the use of this processing aid.
- There is nothing to indicate, at this stage, that the costs, that would arise from a variation to the Code approving the new enzyme, outweigh the direct and indirect benefits to the community, Government or industry of the approval.

The Application is recommended for further consideration, so FSANZ now seeks submissions to assist it in assessing the Application.