



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

4-07

8 August 2007

INITIAL ASSESSMENT REPORT

APPLICATION A606

ASPARAGINASE AS A PROCESSING AID (ENZYME)

DEADLINE FOR PUBLIC SUBMISSIONS: 6pm (Canberra time) 19 September 2007

**SUBMISSIONS RECEIVED AFTER THIS DEADLINE
WILL NOT BE CONSIDERED**

(See 'Invitation for Public Submissions' for details)

For Information on matters relating to this Assessment Report or the assessment process generally, please refer to <http://www.foodstandards.gov.au/standardsdevelopment/>

Executive Summary

An Application was received by Food Standards Australia New Zealand (FSANZ) on 30 April 2007 from Novozymes A/S Denmark (submitted by Novozymes Australia Pty Ltd) seeking the approval of a new enzyme, asparaginase, as a processing aid. Asparaginase is produced from a strain of the host micro-organism *Aspergillus oryzae* expressing the *A. oryzae* asparaginase gene.

Application A606 seeks to amend Standard 1.3.3 – Processing Aids of the *Australia New Zealand Food Standards Code* (the Code) to approve an asparaginase enzyme preparation, (EC number [3.5.1.1]), as a processing aid. The enzyme is proposed for use in food processing to convert the amino acid asparagine to aspartic acid to reduce acrylamide formation during processing of products such as potato chips and French fries and wheat dough based products such as biscuits and crisp breads. The enzyme is produced by submerged fermentation of an *A. oryzae* micro-organism expressing the *A. oryzae* asparaginase gene.

Acrylamide is formed as a reaction product between asparagine and reducing sugars contained in the food when heated above 120°C during baking or frying. Concerns about dietary exposure to acrylamide had arisen as a result of studies conducted in Sweden in 2002, which showed high levels of acrylamide were formed during the frying or baking of a variety of foods. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviewed the safety of acrylamide in 2005 and recommended that acrylamide be re-evaluated when results of ongoing carcinogenicity and long term neurotoxicity studies, which are being conducted around the world, become available and that appropriate efforts to reduce acrylamide concentrations in food should continue.

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.

The objective of the assessment is to determine whether the Code should be amended to permit the use of asparaginase from the micro-organism *A. oryzae* containing the gene coding for asparaginase from *A. oryzae*.

The enzyme preparation meets the international specifications for enzymes, namely the Food Chemicals Codex (5th Edition, 2004) and the 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 (Generally Recognised As Safe) 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 GRAS.

The summary report (GRAS Notice No. GRN 000201, 24 November 2006) from the US Food and Drug Administration (FDA) of this evaluation is provided in the Application.

Purpose

The Application is seeking the approval of a new enzyme, asparaginase, which has a microbial source being *A. oryzae* expressing the asparaginase gene from *A. oryzae*. The asparaginase enzyme is used to reduce acrylamide formation in some foods during processing.

Reasons for Assessment

After considering the requirements for Initial Assessment as prescribed in section 13 of the *Food Standards Australia New Zealand Act 1991* (FSANZ Act), FSANZ has decided to accept the Application for the following reasons:

- The Application seeks approval for a new processing aid, the enzyme asparaginase sourced from *A. oryzae* expressing the *A. oryzae* asparaginase gene. Such an approval, if accepted, would warrant a variation to Standard 1.3.3 – Processing Aids.
- There is currently no permission in the Code for asparaginase sourced from *A. oryzae* expressing the *A. oryzae* asparaginase gene.
- The Application is not so similar to any previous application that it ought not be accepted.
- There are no other measures that would be more cost-effective than a variation to Standard 1.3.3 that could achieve the same end.
- At this stage no other relevant matters are apparent.

Consultation

Public submissions are now invited on this Initial Assessment Report. Comments may be made on any aspects of the Application, though of particular interest will be information related to the safety of the new enzyme and the potential benefits and costs resulting from the proposed regulatory options.

Responses to the Initial Assessment Report will assist in preparing a Draft Assessment of this Application.

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INVITATION FOR PUBLIC SUBMISSIONS

FSANZ invites public comment on this Initial Assessment 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 of 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

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

Submissions need to be received by FSANZ by 6pm (Canberra time) 19 September 2007.

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.

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.

INTRODUCTION

This Application was received from Novozymes A/S (submitted by Novozymes Australia Pty Limited) on 30 April 2007 seeking to vary the *Australia New Zealand Food Standards Code* (the Code). The proposed variation to Standard 1.3.3 – Processing Aids would permit the enzyme asparaginase (also called L-asparagine amidohydrolase) (EC 3.5.1.1), as a processing aid. Asparaginase is produced using recombinant DNA techniques, from a strain of the host micro-organism *Aspergillus oryzae* expressing the *A. oryzae* asparaginase gene.

The Applicant claims that the enzyme hydrolyses the amino acid asparagine to aspartic acid by hydrolyzing the amide in asparagine to the corresponding acid, aspartic acid. It is claimed that the enzyme is intended for use as a processing aid during food manufacture to convert asparagine to aspartic acid to reduce acrylamide formation in baked or fried wheat dough based products such as biscuits and crackers and cut vegetable products such as sliced potato chips and French fries.

Acrylamide is formed as a reaction product between the amino acid asparagine and reducing sugars contained in the food when heated above 120°C during baking or frying.

1. Background

1.1 Current Standard

Standard 1.3.3 regulates the use of processing aids in food manufacture, prohibiting their use unless there is a specific permission in the Standard. There are currently no permissions in Standard 1.3.3 for use of asparaginase as a processing aid in manufacturing food products. Processing aids not permitted in the Code may not be used for food manufacture until there has been a pre-market assessment of their use. This Initial Assessment Report sets out the background to the Application and is used primarily to stimulate useful input from stakeholders by raising issues and asking questions.

Clause 1 of Standard 1.3.3 defines a processing aid as:

processing aid means a substance listed in clauses 3 to 18, where –

- (a) *the substance is 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; and*
- (b) *the substance is used in the course of manufacture of a food at the lowest level necessary to achieve a function in the processing of that food, irrespective of any maximum permitted level specified.*

The Applicant has requested that, if approved, the permission for use of the enzyme be included in the Table to clause 17 – Permitted enzymes of microbial origin as asparaginase EC 3.5.1.1 with the source being *Aspergillus oryzae* expressing the *A. oryzae* asparaginase gene. Under clause 17, the processing aids listed in the Table to this clause may be used as enzymes in the course of manufacture of any food provided the enzyme is derived from the corresponding source or sources specified in the Table.

1.2 Basis of Application

The Applicant proposes introducing asparaginase as a processing aid to be added to food products during processing to convert L-asparagine to L-aspartate and ammonia to reduce the quantity of acrylamide formed during production of products such as potato chips and French fries and wheat dough based products such as biscuits and crisp breads. Acrylamide is formed as a reaction product of asparagine and reducing sugars when food products are baked or fried at temperatures above 120°C. Both asparagine and reducing sugars are commonly found in the ingredients of many food products. The Applicant claims that by using asparaginase, the asparagine content will be reduced, resulting in reduced acrylamide formation and consequently a reduced acrylamide content in the final product benefiting consumers by decreasing acrylamide intake through food products.

1.3 Acrylamide in Food

JECFA undertook an evaluation of acrylamide at its sixty-fourth meeting, at the request of the Codex Committee on Food Additives and Contaminants (JECFA 2005)¹. The Committee had not previously evaluated acrylamide. Concerns about dietary exposure to acrylamide had arisen as a result of studies conducted in Sweden in 2002, which showed high levels of acrylamide were formed during the frying or baking of a variety of foods. JECFA recommended that acrylamide be re-evaluated when results of ongoing carcinogenicity and long term neurotoxicity studies become available and that appropriate efforts to reduce acrylamide concentrations in food should continue.

In April 2007, the Codex Committee on Contaminants in Food (CCCF) commenced work on a draft Code of Practice for the Reduction of Acrylamide in Food². This document flags the potential use of the enzyme asparaginase to reduce asparagine and hence acrylamide formation in food, specifically potato products made from potato doughs and cereal-based products.

1.4 Nature and Technological Justification of the Enzyme

The systematic name of the enzyme is L-asparagine amidohydrolase, and the accepted name is asparaginase³. Other names include asparaginase II, L-asparaginase, colaspase, elspar, leunase, crasnitin and α -asparaginase.

The enzyme has the Enzyme Commission (EC) number of 3.5.1.1 and a Chemical Abstracts Service (CAS) number of 9015-68-3. It is referred to by its generic name asparaginase in this report.

The enzyme preparation is a clear to pale yellow water soluble liquid. The enzyme is stable between pH 5.0 to 9.0. The enzyme activity range occurs between pH 5.0 to 9.0, with its optimum activity at pH 7.0. The optimum temperature of use is 60°C. The molecular weight of the enzyme was determined to be 36 kDa.

¹ Joint FAO/WHO Expert Committee on Food Additives (JECFA) *Report on 64th meeting* (Rome, 8-17 February 2005), Acrylamide, pages 7-17,

http://www.who.int/ipcs/food/jecfa/summaries/summary_report_64_final.pdf, accessed on 4 June 2007

² Codex Committee on Contaminants in Foods (Beijing 16-20 April 2007) Proposed Draft Code of Practice for the Reduction of Acrylamide in Food, at ftp://ftp.fao.org/codex/cccf1/cf01_15e.pdf Accessed on 3 July 2007

³ International Union of Biochemistry and Molecular Biology (IUBMB) Enzyme Nomenclature <http://www.chem.qmul.ac.uk/iubmb/enzyme/EC3/5/1/1.html>, accessed on 30 April 2007

This asparaginase is produced by submerged fermentation of an *A. oryzae* strain expressing the *A. oryzae* asparaginase gene inserted by recombinant DNA techniques. The secreted asparaginase is recovered from the fermentation medium, concentrated and stabilised. The resultant enzyme preparation is formulated, stabilised and standardised using water, glycerol, sodium benzoate and potassium sorbate, which are all commonly used and approved stabilisers for enzyme preparations. The Applicant claims the enzyme preparation contains no free production organism and meets the specifications of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)⁴ and Food Chemicals Codex⁵ for enzyme preparations.

Asparaginase hydrolyses the amino acid asparagine to aspartic acid by hydrolysing the amide in free asparagine to the corresponding acid, aspartic acid. Apart from asparagine, asparaginase only acts on glutamine and has no activity on other amino acids. It has no activity on asparagine residues in peptides or proteins.

The technological justification of the enzyme will be investigated more fully at Draft Assessment.

1.5 International Permissions

The Applicant submitted a self-affirmed GRAS (generally recognised as safe) notification (GRAS Notice No. GRN 000201) for this same enzyme to the US Food and Drug Administration (FDA) for which it received the ‘no questions letter’ on November 2006. This information is contained in the Application, while the FDA letter can be obtained from the FDA website⁶.

The Applicant states that the enzyme can already be legally sold in Germany, Great Britain, Italy, Ireland and the USA. An application seeking approval for the enzyme has been submitted to JECFA and Denmark and will be submitted in France also in 2007.

There is no Codex standard for the enzyme, since there are no specific Codex standards for enzymes. However the Applicant states that the enzyme complies with the specifications for enzymes of both JECFA and the Food Chemicals Codex.

JECFA also examined the same enzyme from the same source micro-organism (and the same information as contained in the Application) at their sixty-eight meeting, 19-28 June 2007 in Geneva with the summary report available. This summary report indicated that the enzyme had an “ADI of ‘not specified’ when use in the applications specified and in accordance with good manufacturing practice”⁷.

⁴ 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.

⁵ Food Chemicals Codex (2004), National Academy of Sciences, Food and Nutrition Board, Committee on Food Chemical Codex, 5th Edition, National Academy press, Washington DC, pp146-152.

⁶ <http://www.cfsan.fda.gov/~rdb/opa-g201.html>

⁷ Joint FAO/WHO Expert Committee on Food Additives, Sixty-eight meeting, Summary and Conclusions, 19-28 June 2007, Geneva, <http://www.who.int/ipcs/food/jecfa/summaries/summary68.pdf> accessed on 16 July 2007.

2. The Issue / Problem

Processing aids (which includes enzymes) are required to undergo a pre-market assessment before they are approved for use in food manufacture.

The Table to clause 17 of Standard 1.3.3 contains a list of permitted enzymes of microbial origin. There is currently no permission for the enzyme asparaginase, from any source in this Table. Therefore an assessment (which includes a safety assessment) of the use of the enzyme is required before it can be approved or used.

3. Objectives

The objective of this assessment is to determine whether it is appropriate to amend the Code to permit the use of asparaginase from the source, *A. oryzae* expressing the *A. oryzae* asparaginase gene.

In developing or varying a food standard, FSANZ is required by its legislation to meet three primary objectives which are set out in section 18 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. Key Assessment Questions

The key question which FSANZ needs to consider as part of this assessment, in particular at Draft Assessment is:

- Are there any public health and safety issues with approving the asparaginase enzyme sourced from *A. oryzae* expressing the *A. oryzae* asparaginase gene?

RISK ASSESSMENT

5. Safety assessment

A key part of the pre-market evaluation process for a new enzyme processing aid involves consideration of the safety of the enzyme for use in food. This includes an evaluation of the source of the enzyme, the purified enzyme preparation, and the potential for the enzyme preparation to contain contaminants.

The host micro-organism *A. oryzae*, is stated by the Applicant to be non-pathogenic and has a long history of safe use in food. It is the source organism for a number of approved enzymes, including enzymes produced using recombinant DNA technology, listed in the Table to clause 17 of Standard 1.3.3.

The Applicant believes the genetic modifications to produce the enzyme source are well characterised, utilising well-known plasmids for the vector constructs. The Applicant states that because the introduced genetic material does not encode any known harmful or toxic substances, it is considered a safe source. Data to support this conclusion have been submitted and will be considered by FSANZ at Draft Assessment.

In addition, the Applicant has provided the following studies on the asparaginase enzyme:

- A 13-week sub-chronic oral toxicity study in rats;
- A test for mutagenic activity (Ames test); and
- A human lymphocyte cytogenetic assay.

These studies will be assessed as part of the safety assessment of asparaginase.

This enzyme and source were evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at their recent meeting in June 2007. An ADI of 'not specified' was allocated and a specification prepared. The FSANZ safety assessment will consider the findings of JECFA.

The Application contains information and data dealing with the reduction in levels of acrylamide in food produced using the enzyme. This information will be assessed as part of the Draft Assessment. There will only be minor levels of any enzyme (inactivated by heat to inactive protein) in the final food as consumed. The Applicant did, however, provide a dietary exposure estimate for the enzyme noting the enzyme deactivation. Overall, there are not expected to be any significant dietary exposure considerations, so it is anticipated that no dietary exposure assessment will be conducted by FSANZ on the asparaginase enzyme itself.

RISK MANAGEMENT

6. Options

FSANZ is required to consider the impact of various regulatory (and non-regulatory) options on all sections of the community, especially relevant stakeholders who may be affected by this Application. The benefits and costs associated with the proposed amendment to the Code will be analysed using regulatory impact principles at Draft Assessment.

Enzymes (being processing aids in the Code) used in Australia and New Zealand are required to be listed in Standard 1.3.3, and it is not appropriate to consider non-regulatory options.

Two regulatory options have been identified for this Application:

Option 1 Not permit the use of asparaginase sourced from *A. oryzae* expressing the *A. oryzae* asparaginase gene.

Option 2 Permit the use of asparaginase sourced from *A. oryzae* expressing the *A. oryzae* asparaginase gene 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, including importers of food, wishing to produce and market food products manufactured using this enzyme;
2. consumers; and
3. Government agencies in Australia and New Zealand who enforce the Code.

7.2 Benefit Cost Analysis

In developing food regulatory measures suitable for adopting in Australia and New Zealand, FSANZ is required to consider the impact of all options on affected parties in both countries. The benefit cost analysis 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.

COMMUNICATION AND CONSULTATION STRATEGY

8. Communication

It is considered that this Application will be a routine matter. Therefore, FSANZ has applied a basic communication strategy. This will involve advertising the availability of assessment reports for public comment in the national press and making reports available on the FSANZ website.

The Applicant and individuals and organisations who make submissions on this Application will be notified at each stage of the assessment of the Application. If approval is recommended, once the FSANZ Board has approved the Final Assessment Report, FSANZ will notify the Ministerial Council. The Applicant and stakeholders, including the public, will be notified of the gazettal of changes to the Code in the national press and on the website.

FSANZ provides an advisory service to the jurisdictions on changes to the Code.

9. Consultation

Public comment on this Initial Assessment Report is sought to assist in completing the Draft Assessment.

The purpose of the Initial Assessment Report is to seek early input on a range of specific issues known to be of interest to various stakeholders relevant to the assessment of this Application.

All stakeholders that make a submission in relation to the Application will be included on a mailing list to receive further FSANZ documents in relation to the Application. If readers of the Initial Assessment report know of other stakeholders who might have an interest in this Application, they should bring it to their attention. Other interested parties, as they come to the attention of FSANZ, will also be added to the mailing list for public consultation even if they do not provide a submission.

FSANZ is seeking public comment to assist it complete the assessment of this Application. All stakeholders must observe the relevant due date for submissions.

FSANZ seeks comment on this Application which include, but is not exclusive to, the following issues:

- The safety of asparaginase sourced from *Aspergillus oryzae* expressing the *A. oryzae* asparaginase gene as an enzyme used for food manufacture.
- The foods and food industries that may use the asparaginase enzyme to potentially reduce the formation of acrylamide in the final food.

9.2 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.

There are not any relevant international standards for processing aids or specifically enzymes and amending the Code to allow permission to use asparaginase sourced from *A. oryzae* containing the gene for asparaginase from *A. oryzae* is unlikely to have a significant effect on international trade. The enzyme preparation is consistent with the international specifications for food enzymes of JECFA and the Food Chemicals Codex 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 Barriers to Trade (TBT) or Sanitary and Phytosanitary Measures (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.

CONCLUSION

10. Conclusion

After considering the requirements for Initial Assessment as prescribed in section 13 of the FSANZ Act, FSANZ has decided to accept the Application for the following reasons:

- The Application seeks approval for a new processing aid, the enzyme asparaginase sourced from *A. oryzae* expressing the *A. oryzae* asparaginase. Such an approval, if accepted, would warrant a variation to Standard 1.3.3 – Processing Aids.
- There is currently no permission in the Code for asparaginase sourced from *A. oryzae* expressing the *A. oryzae* asparaginase.
- The Application is not so similar to any previous application that it ought not be accepted.
- There are no other measures that would be more cost-effective than a variation to Standard 1.3.3 that could achieve the same end.
- At this stage no other relevant matters are apparent.