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

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[17-08]

APPLICATION A1003

ASPARAGINASE FROM *ASPERGILLUS NIGER* AS A PROCESSING AID (ENZYME) APPROVAL REPORT

Executive Summary

Purpose

Food Standards Australia New Zealand (FSANZ) received an Application from DSM Food Specialties (DSM) on 4 February 2008. The Application 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), produced from a strain of the host micro-organism *Aspergillus niger* expressing the *A. niger* asparaginase gene, as a processing aid.

Asparaginase hydrolyses the amino acid, L-asparagine, to L-aspartic acid, thus preventing the asparagine from reacting with reducing sugars to form acrylamide. The asparaginase enzyme is proposed for use as a processing aid to reduce acrylamide formation during the frying or baking process of potato based products such as potato chips and French fries, wheat dough based products such as biscuits and crisp breads, and yeast reaction flavours. All the intended applications involve heating foods at temperatures well above the inactivation temperature of the enzyme (around 70^{o}C) therefore no active enzyme is expected to remain in the product.

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. Different mitigating methods are currently being evaluated to reduce acrylamide formation and reviews show that the application of asparaginase prior to the heating step in heat processes is beneficial to reduce acrylamide formation in some foods. The additional benefit of this treatment is that no further adjustment to the formulation and process is needed, therefore maintaining the sensory properties of the products.

Processing aids are required to undergo a pre-market safety assessment before approval for use in Australia and New Zealand. There is currently an approval in the Code for asparaginase sourced from an alternative source, *Aspergillus oryzae*.

The enzyme preparation meets the international specifications for enzymes. The US Food and Drug Administration (FDA) had no objections to the enzyme being notified as GRAS (Generally Recognized As Safe) (filed 11 October 2006).

Both Denmark and Russia have approved the product. The French food safety authority, AFSSA (Agence Française de Sécurité Sanitaire des Aliments), has endorsed the safety of the enzyme.

Asparaginase from *A. niger* was evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at its meeting in June 2008, with an Acceptable Daily Intake (ADI) of 'not specified' being established.

When processing aid enzymes are produced from genetically modified (GM) micro-organism sources, the enzymes in this case do not contain novel proteins since they are identical to other enzymes sourced from non-GM sources. The refinement process the enzyme preparation undergoes removes all the source organisms from the preparation so there is no novel DNA remaining in the enzyme preparation. This is the case for a number of enzymes sourced from GM micro-organisms approved in the Code.

The Application has been assessed under the General Procedure.

Safety Assessment

The enzyme is used as a processing aid only, and any residue consumed would be in the form of inactivated enzyme, which would be metabolised like any other protein.

The hazard assessment concluded that:

- there was no evidence of toxicity in the 90-day toxicity study or a developmental study in rats;
- the NOEL (no observed effect level) from the 90-day toxicity study was greater than 1157 mg/kg bw per day, the highest tested dose level. This is equivalent to 1038 mg TOS (total organic solids)/kg bw/day;
- the enzyme preparation gave no evidence of any genotoxic potential in *in vitro* assays; and
- there was no evidence of any immunologically significant amino acid sequence homology with known allergens.

Based on the available evidence it is concluded that no hazard has been identified for asparaginase derived from this recombinant strain of *A. niger*. In view of the acid lability and sensitivity to proteolytic digestion following oral ingestion of asparaginase the absence of a hazard may have been reasonably anticipated. The ADI can be considered to be 'not specified'. Because of this, there was no need to perform a dietary exposure assessment relating to the asparaginase enzyme use in food manufacture.

Labelling

The enzyme preparations may contain either maltodextrin sourced from wheat or wheat flour as fillers for standardising the product to an enzyme activity of 2,500 ASPU (asparaginase units)/g.

The Applicant provides an allergen statement in the product specification. Therefore their customers will have to declare the presence of these allergens in the final products on the label of any food produced using such forms of the enzyme (under the labelling requirements of clause 4 of Standard 1.2.3 – Labelling of Ingredients).

Assessing the Application

In assessing the Application and the subsequent development of a food regulatory measure, FSANZ has had regard to the following matters as prescribed in section 29 of the *Food Standards Australia New Zealand Act 1991* (FSANZ Act):

- whether costs that would arise from an amendment to the Code to permit the use of the enzyme asparaginase sourced from *Aspergillus niger* expressing the *A. niger* asparaginase gene would outweigh the direct and indirect benefits to the community, Governments or industry;

- 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;

- there are no relevant New Zealand standards;

- there are no other relevant matters.

Decision

FSANZ approves the draft variation to the Table to clause 17 of Standard 1.3.3 – Processing Aids, to permit the use of the enzyme asparaginase sourced from *Aspergillus niger* expressing the *A. niger* asparaginase gene.

Reasons for Decision

An amendment to the Code approving the use of the asparaginase enzyme as a processing aid in Australia and New Zealand is approved on the basis of the available scientific evidence for the following reasons:

- A detailed safety assessment has concluded that the use of enzyme does not raise any public health and safety concerns.

- Use of the enzyme is technologically justified as a treatment to reduce the formation of acrylamide in some foods.

- The impact analysis concluded that the benefits of permitting the use of the enzyme to reduce the formation of acrylamide in some treated foods outweigh any associated costs.

- The proposed variation is consistent with the section 18 objectives of the FSANZ Act.

Consultation

Public submissions were invited on the Assessment Report between 4 June and 16 July 2008. Comments were specifically requested on the scientific aspects of this Application, in particular, information relevant to the safety assessment of the enzyme asparaginase from *A. niger* expressed in *A. niger*.

A total of five submissions were received. A summary of these is provided in **Attachment 2** to this Report.

As this Application is being assessed under the General Procedure, there was one round of public comment. Responses to the Assessment Report were used to develop this Approval Report. The main issues raised in public comments are discussed in this Report.

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INTRODUCTION

An Application was received from DSM Specialties (The Netherlands) on 4 February 2008 seeking an amendment of the *Australia New Zealand Food Standards Code* (the Code) to Standard 1.3.3 – Processing Aids. The proposed variation to Standard 1.3.3 would permit the enzyme asparaginase to be used as a processing aid. The asparaginase is produced from a genetically modified strain of *Aspergillus niger* expressing the *A. niger* asparaginase gene.

The Applicant claims that the enzyme hydrolyses the amino acid asparagine to aspartic acid, thus reducing the amount of asparagine in potato and wheat starch foods. Asparagine is one of the precursors of the Maillard browning reaction, reacting with reducing sugars to form acrylamide during high temperature manufacturing processes. Therefore, treating food to reduce the concentration of asparagine prior to heat processing would reduce the amount of acrylamide in foods such as potato chips, bread and reaction flavours.

1. The Issue / Problem

The Applicant proposed the use of the asparaginase as a processing aid. 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 permission in the Code for asparaginase sourced from an alternative source, *A. oryzae*. An assessment (which includes a safety assessment) of the use of the enzyme sourced from *A. niger* is required before it can be approved or used.

2. Current Standard

2.1 Background

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 is currently permission in Standard 1.3.3 for the use of asparaginase sourced from *A. oryzae* for use as a processing aid in manufacturing food products, but not currently for *A. niger.*

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 *A. niger* expressing the *A. niger* 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.

2.2 Basis of Application

The Applicant proposes to use the asparaginase enzyme as a processing aid to reduce the formation of acrylamide when high temperatures are used to process high starch foods. Asparaginase converts asparagine into aspartic acid, thus preventing the asparagine, which is one of the precursors of the Maillard browning reaction, reacting with reducing sugars to form acrylamide.

2.3 Acrylamide in food

Acrylamide is formed by the heat-induced reaction between a reducing sugar and asparagine, which is one of the reaction pathways of the Maillard reaction. The Maillard reaction is the process that gives the brown colour and tasty flavour of baked, fried and toasted foods.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) undertook an evaluation of acrylamide at its 64th 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.

The Confederation of the Food and Drink Industries of the EU (CIAA, Confédération des Industries Agro-Alimentaires de l'UE) produced an Acrylamide 'Toolbox' in 2007 (revision 11)[2] to assist the food industry to utilise methods to minimise the formation of acrylamide in their processed food. It specifically mentions using asparaginase in food processing, with the understanding that regulatory approval is first required.

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 Food[3]. This document highlights the potential use of the enzyme asparaginase to reduce asparagine and hence acrylamide formation in food, specifically potato products made from potato dough and cereal-based products.

2.4 Nature of the Enzyme and Source of Organism

The systematic name of the enzyme is L-asparagine amidohydrolase, and the accepted name is asparaginase which is the name used in this Report.

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[1] Joint FAO/WHO Expert Committee on Food Additives (JECFA) *Report on 64th meeting* (Rome, 8-17 February 2005), Acrylamide, pp 7-17, http://www.who.int/ipcs/food/jecfa/summaries/summary_report_64_final.pdf, Accessed on 19 September 2007

[2] CIAA, 2007. The CIAA acrylamide 'Toolbox'-Rev.11. http://www.ciaa.be/documents/brochures/toolbox%20rev11%20nov%202007final.pdf, Accessed 07 August 2008

[3] 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/cf 01_15e.pdf, Assessed on 19 September 2007

The commercial names of the DSM asparaginase preparation are PreventASe™ M and PreventASe™ W for the spray-dried forms containing maltodextrin and wheat flour, respectively, and PreventASe™ L for the liquid form standardised with glycerol.

The enzyme has the Enzyme Commission (EC) number of 3.5.1.1 and a Chemical Abstracts Service (CAS) number of 9015-68-3.

The enzyme activity range occurs between pH 4.0 to 8.0, with its optimum activity at pH 4.0-5.0. The optimum temperature of use is 50ºC and the enzyme is inactivated at 70ºC. The molecular weight of the protein part of the enzyme was determined to be about 39.6 kDa.

The Application indicates that the source micro-organism is a genetically modified selected strain of *A. niger* which contains extra copies of the asparaginase gene obtained from *A. niger*. The extra copies of the asparaginase gene inserted into the source micro-organism improve the yield of the enzyme during fermentation. The Applicant confirmed that the production strain does not produce any known toxins under conditions which favour toxin synthesis.

FSANZ has finalised and granted permission for use of another form of the asparaginase enzyme sourced from a genetically modified micro-organism (*A. oryzae* expressing the asparaginase gene from *A. oryzae*) produced by Novozymes A/S Denmark (Application A606). FSANZ finalised its assessment of A606 in April 2008 and it was gazetted in amendment 100 (10 July 2008) of the Code.

Both enzymes, from A1003 and A606, are produced from microbial sources but differ in their strains, *A. niger and A. oryzae*, respectively. The success of one application does not provide permission to use the other. A separate pre-market assessment is required for each enzyme before a separate permission could be granted. The Table to clause 17 of Standard 1.3.3 provides individual permissions to enzymes derived from specific source micro-organisms, so the permission is source-specific.

2.2 International Permissions

Asparaginase from *A. nige*r has been notified as Generally Recognized As Safe (GRAS) in the USA (GRN 000214). A no-questions letter from the Food and Drug Administration (FDA) was sent on 12 March 2007. The enzyme has been evaluated by the French Food Safety Authority (Agence Française de Sécurité Sanitaire des Aliments; AFSSA) and a positive advice was issued on 31 May 2007. The use of the enzyme has been approved in Denmark and Russia on 24 September 2007 and 30 August 2007, respectively. A dossier for acceptance has been filed in Mexico and Switzerland. In the remaining European countries, there are no legal restrictions to use enzymes as processing aids in food.

Asparaginase from *A. niger* was evaluated by the Joint FAO/WHO Expert Committee on Food Additives at its meeting in 2008. New specifications were prepared and published in the FAO JECFA Monographs 5 (2008), with an Acceptable Daily Intake (ADI) of 'not specified' being established[4].

3. Objectives

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:

[4] JECFA (2008) Asparaginase from *Aspergillus niger expressed in A. niger*, at http://www.fao.org/ag/agn/jecfa-additives/specs/monograph5/additive-504-m5.pdf, Assessed on 07 August 2008

- 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[5].

4. Questions to be answered

The key question which FSANZ considered as part of the assessment was:

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

RISK ASSESSMENT

5. Risk Assessment Summary

5.1 Safety Assessment

A safety assessment was conducted as part of this Application (**Attachment 3**).

The safety assessment concluded the following:

- Asparaginase from *A. niger* is used as a processing aid only, and any residue would be in the form of inactivated enzyme, which would be metabolised like any other protein.
- There was no evidence of toxicity in the 90-day toxicity study or a developmental study in rats.
- The NOEL (no observed effect level) from the 90-day toxicity study was greater than 1157 mg/kg bw per day, the highest tested dose level. This is equivalent to 1038 mg TOS (total organic solids)/kg bw/day.
- The enzyme preparation gave no evidence of any genotoxic potential in *in vitro* assays.

[5] In May 2008 the Australia and New Zealand Food Regulation Ministerial Council endorsed the Policy Guideline on Addition to Food of Substances other than Vitamins and Minerals. This includes policy principles in regard to substances added for technological purposes such as food additives and processing aids. FSANZ has given regard to each of these principles in assessing this application

- There was no evidence of any immunologically significant amino acid sequence homology with known allergens.

Based on the available evidence it is concluded that for asparaginase derived from this recombinant strain of *A. niger* no hazard has been identified. In view of the acid lability and sensitivity to proteolytic digestion following oral ingestion of asparaginase, the absence of a hazard may have been reasonably anticipated. The ADI can be considered to be 'not specified'.

5.2 Dietary Exposure Assessment of Asparaginase

FSANZ reviewed the dietary exposure assessment for the enzyme asparaginase provided by the Applicant. The Applicant's estimate of the dietary exposure to asparaginase was based on the Budget Method and the Estimated Daily Intake (EDI) for the Netherlands from which margins of safety were calculated. The Budget Method is an internationally accepted methodology used to screen food additives for safety concerns using very conservative assumptions and for which there is an Acceptable Daily Intake(ADI) (Hansen, 1979)[6]. Taking into account that asparaginase is allocated 'ADI not specified' and is acid and protease labile in the gastro-intestinal tract so presenting no systemic exposure, FSANZ considers a dietary exposure assessment for asparaginase was unnecessary.

5.3 Technological Justification

A full technical report on the technological function of the enzyme is provided in **Attachment 4**. Reviews on studies using different mitigating methods to reduce acrylamide show that the application of asparaginase to food prior to the heating step in heat processes is promising. The enzyme is inactivated at temperatures above 70^{o}C and becomes a residual protein. Thus no enzyme activity is expected to remain in the finished products because all the intended applications involved heating above this temperature, including the interior of baked bread. The Applicant verified this expectation on baked bread and yeast extract/reaction flavour samples and showed no asparaginase activity is present in the final products.

The additional benefit of this treatment is that no further adjustment to the formulation and process is needed, therefore maintaining the sensory properties of the products. Results of trials reported by the Applicant on the efficacy of their asparaginase enzyme preparation showed a reduction in acrylamide ranging from about 35 to 100% in the final food products. Their findings are supported by other researchers in the literature as cited in **Attachment 4**.

Overall, the use of the asparaginase enzyme sourced from *Aspergillus niger* expressing the *A. niger* asparaginase gene as a processing aid is technologically justified to treat food products such as breads and bakery products, cereal-based and potato-based products and reaction flavours, which are subjected to high heat, to reduce the formation of acrylamide in the final products.

5.4 Production of the enzyme

The Applicant states that the asparaginase enzyme is produced by a controlled submerged, two-step, fermentation process (the fermentation medium consists of glucose, yeast extract and antifoaming agent). Once the fermentation has been completed the production organism is killed off by incubating with sodium benzoate at pH 4.0 to 4.5 for 10 hours at 30^{o}C.

[6] Hansen, S.C. (1979). Conditions for use of food additives based on a budget for an acceptable daily intake. *Food Protect* 42(5):429-432.

The desired enzyme is separated from the microbial biomass using simple filtrations (broth filtration, followed by polish filtration and a germ reduction filtration) and then the enzyme is concentrated by ultra-filtration (UF). The enzyme preparation in liquid form is standardised and stabilised by adding glycerol to give a final enzyme activity of 2500 ASPU (asparaginase units)/ml. The dry enzyme preparation is obtained by spray drying the UF concentrate and standardised with either maltodextrin or wheat flour to an activity of 2500 ASPU/g.

Glycerol or glycerine (INS 422) is listed in Schedule 2 of Standard 1.3.1 as a food additive and is approved in many processed foods to levels determined by Good Manufacturing Practice. Schedule 2 additives are also generally permitted processing aids. Sodium benzoate (INS 211) is a permitted preservative in a number of foods specified in Schedule 1 of Standard 1.3.1. There are no specific requirements for food additives for the enzyme preparations in the Code.

5.5 Allergenicity

Given the manufacturing process and ingredients described above, no allergenic materials (given in the Table to clause 4, Standard 1.2.3) are likely to be present, except when wheat flour or maltodextrin sourced from wheat is used as fillers in the dry enzyme preparation. In cases where wheat flour or maltodextrin sourced from wheat is used, it should be declared on the label of any food that has been treated with the asparaginase enzyme preparation.

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 have been analysed using regulatory impact principles.

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 Reject the Application

Option 2 Permit the use of asparaginase sourced from *A. niger* as a processing aid.

7. Impact Analysis

In developing food regulatory measures 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 relevant food industries and governments. The regulatory impact assessment identifies and evaluates, though is not limited to, the costs and benefits arising from the regulation and its health, economic and social impacts. The regulatory impact analysis is designed to assist in the process of identifying the affected parties and the likely or potential impacts the regulatory provisions will have on each affected party. Where medium to significant competitive impacts or compliance costs are likely, FSANZ will seek further advice from the Office of Best Practice Regulation to estimate compliance costs of regulatory options.

7.1 Affected Parties

The affected parties to this Application include the following:

- consumers of foods high in starch and processed under high heat
- food industry, including importers of food, wishing to produce and market food products manufactured using this enzyme
- the Governments of Australia (Federal, State and Territory) and New Zealand

7.2 Benefit Cost Analysis

7.2.1 Option 1 – Reject the Application

There are no perceived benefits to the food industry, consumers or government agencies if this option is progressed.

Rejecting the Application would disadvantage consumers and relevant food industries where the enzyme could reduce the formation of acrylamide in their products. It could also leave government agencies open to criticism that not all viable treatments to reduce the formation of acrylamide in food have been investigated and supported.

7.2.2 Option 2 – Permit the use of asparaginase sourced from A. niger as a processing aid

This option does provide benefits to consumers, the food industry and indirectly to government agencies. The asparaginase enzyme has been developed and assessed to reduce the formation of acrylamide in some processed foods so assisting in reducing the levels of this compound in the food supply of consumers. It also provides some food industries a viable commercial method to reduce the formation of acrylamide without compromising the quality, flavour or characteristics of their processed food. At the same time, government agencies are able to indicate to international agencies (specifically JECFA) that they are assisting the food industry in developing procedures to reduce the formation of acrylamide in the food supply.

There should not be any significant compliance costs for government enforcement agencies since they would not need to analyse for the presence of the enzyme, nor would it be expected that they would need to analyse for acrylamide due to this Application. If acrylamide analyses in food will be required in the future, it should not be as a result of this Application.

7.3 Comparison of Options

Option 2 is favoured since there is no benefit derived for any affected party for Option 1, while consumers, relevant food industries and government agencies would all be advantaged by adopting Option 2.

The outcome of approving the use of this asparaginase enzyme as a processing aid can aid in reducing the formation of acrylamide in some processed food products.

7.4 Drafting name for microbial source organism

To give effect to option 2, giving permission for the enzyme, required an assessment of how to incorporate the enzyme and the source micro-organism into the Code. Approved enzymes from microbial sources are listed in the Table to clause 17 of Standard 1.3.3, so an entry for the enzyme in this Table is required.

Subclause 17(2) of Standard 1.3.3 states that:

> *The sources listed in the Table to this clause may contain additional copies of genes from the same organism.*

This is the situation for asparaginase derived from *A. niger.* Therefore, the source micro-organism can be simply given as *Aspergillus niger*. The draft variation is provided in **Attachment 1**.

COMMUNICATION AND CONSULTATION STRATEGY

8. Communication

It is considered that this Application is a routine matter. Therefore FSANZ has applied a basic communication strategy to this Application that involves advertising the availability of assessment reports for public comment in the national press and placing the reports on the FSANZ website.

Public comment on the Assessment was sought prior to preparation of this Approval Report.

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

9. Consultation

9.1 Issues Raised in Public Consultation

The Assessment Report was advertised for public comment between 4 June and 16 July 2008. Comments were specifically requested on the scientific aspects of this Application. As this Application is being assessed under a General Procedure, there was one round of public comment.

A total of five submissions were received, three submissions supported the Application, one requested issues that they would like to be addressed at the Approval stage and one opposed the Application due to its GM aspects. A summary of these is provided in **Attachment 2** to this Report.

FSANZ has taken the submitters' comments relevant to food safety into account in preparing the Approval Report for this Application. Specific issues relating to the inactivation of the enzyme in all treated foods and any potential issues relating to enforcement and methodology of testing, these issues are further discussed below. Responses to general issues, such as GM food labelling are available from the FSANZ website[7].

[7] FSANZ (2008) Frequently Asked Questions on Genetically Modified Foods http://www.foodstandards.gov.au/foodmatters/gmfoods/frequentlyaskedquest3862.cfm, Accessed on 12 August 2008

9.1.1 Inactivation of the enzyme in all treated foods

One submission asked about the potential for active residues of the enzyme to be present in the final food product, whereby the food has not undergone sufficient treatment (heating) for inactivation. If so they noted that the enzyme should be treated as a food additive and not as a processing aid.

9.1.1.1 FSANZ response

Any residual asparaginase enzyme is inactivated during the heating process and the subsequent inactivated enzyme is considered to be standard protein.

The inactivation temperature for the asparaginase enzyme is around 70°C, depending on time, pH and the food matrix. The processing of all foods that could be expected to be treated with the asparaginase enzyme will be treated at temperatures higher than the inactivation temperature so there will be no active enzyme left in the final food. In this case the asparaginase enzyme is considered to be a processing aid, where it has performed its technological function to reduce the formation of acrylamide during the processing of the food, it is inactivated and has no further technological function in the final food.

Furthermore, the formation of acrylamide in food is increased with higher temperature as a reaction product between the amino acid asparagine and reducing sugars. Therefore, the mitigation process of using asparaginase to treat food to limit the formation of acrylamide will be for products which undergo high temperature cooking, which will also inactivate the enzyme after it has performed it technological function.

9.1.2 Issues relating to enforcement

One submission noted that State and Territory enforcement agencies will need to be able to analyse the presence of asparaginase and/or genetically modified *Aspergillus niger*, and be able to determine the amount of acrylamide in food for enforcement purposes. Additionally the Benefit Cost Analysis needs to acknowledge that the enforcement agencies will be responsible for bearing the costs related to periodic monitoring of food products in which asparaginase has been used as well as the costs associated with the investigation into any inappropriate use of asparaginase in the industry.

9.1.2.1 FSANZ response

It is not expected that any particular additional enforcement work would be required. There should not be any asparaginase activity in the final produced foods since the enzyme is inactivated during the heating step. Also, there should be no presence of the production micro-organism (*A. niger*) in the treated food since the production specification requires the absence of the production organism in the enzyme preparation, with all microorganism cells being removed from the preparation at the end of fermentation.

It would be up to individual jurisdictions whether there is the need for analyses for the enzyme, the source micro-organism and/or acrylamide. If such analyses were required then added analytical costs would likely be incurred by the jurisdictions, either to develop capability to perform analyses (methods would be available from the Applicant) or to get them performed by external agencies. The JECFA specification for the enzyme contains an analytical method for determining asparaginase activity[8].

[8] JECFA (2008) Asparaginase from *Aspergillus niger expressed in A. niger*, at http://www.fao.org/ag/agn/jecfa-additives/specs/monograph5/additive-504-m5.pdf, Assessed on 07 August 2008

Analyses for acrylamide in the final food should not be required as part of this Application, though there may well be further acrylamide survey and analyses work required as part of some broader work relating specifically to acrylamide. It would be expected that such analytical work measuring acrylamide levels in food would be undertaken by a commercial laboratory such as National Measurement Institute (NMI, formerly the Australia Government Analytical Laboratories) who undertook the earlier acrylamide analyses for the survey of acrylamide levels in Australian food undertaken in late 2002 and who have the analytical methods available for such measurements[9].

9.1.3 Outcome from JECFA evaluation

One submission noted from the assessment report that JECFA was expected to evaluate asparaginase from *A. Niger* expressed in *A. Niger* at its meeting in June 2008 and requested these outcomes need to be considered in the Approval Report.

9.1.3.1 FSANZ response

FSANZ has noted that an ADI of 'not specified' was established at the 69th JECFA (2008)[10] and has been addressed in the 'International permissions' section 2.5 of this report.

9.1.4 Issues relating to the management of food allergens used as 'fillers' in processing aids

One submission received noted that any enzyme preparations containing fillers utilising allergenic sources such as wheat flour will require an allergen declaration on the label of the final food product produced using such forms of enzymes. They also noted that this issue of using allergens as fillers in enzyme preparations needs to be addressed under the proposed work for the Regulatory Management of Food Allergens.

9.1.4.1 FSANZ response

In accordance with the current clause 4 of Standard 1.2.3, the presence of allergenic substances must be declared on the label of the final food product. These requirements apply to ingredients, additives and processing aids and, therefore, would capture fillers in enzyme preparations. For unpackaged foods, allergens must be declared on or in connection with the display of a food or declared to the purchaser upon request.

9.1.5 Issues relating to the GM aspects of the Application

One submitter objected to the Application on the grounds of the GM aspects of the Application. No other issues were raised, beyond an opposition to GM food or foods containing components derived using GM techniques.

9.1.5.1 FSANZ response

The safety aspects of both the enzyme and its source organism have been thoroughly addressed in **Attachment 3**. In the case of processing aid enzymes produced from genetically modified (GM) micro-organisms, the final enzyme is not a novel protein since it is identical to other enzymes sourced from non-GM sources.

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[9] Croft, M.; Tong, P.; Fuentes, D. and Hambridge, T. (2004) Australian survey of acrylamide in carbohydrate-based foods. *Food Add. Contamin*. 21(8):721-736

[10] JECFA (2008) Summary and Conclusions (69th meeting; 17 – 26 June 2008) http://www.who.int/ipcs/food/jecfa/summaries/summary69.pdf, Assessed on 07 August 2008

The refinement process for the enzyme preparation removes all the source organisms from the preparation so there is no novel DNA in the enzyme preparation. This is the case for a number of enzymes sourced from GM micro-organisms in the Code.

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 no relevant international standards for processing aids or specifically enzymes. Amending the Code to allow permission to use asparaginase sourced from *A. niger* containing additional copies of the *A. niger* gene encoding asparaginase 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.

For these reasons FSANZ did not notify the WTO under either the Technical Barriers to Trade (TBT) or Sanitary and Phytosanitary Measures (SPS) Agreements.

CONCLUSION

10. Conclusion and Decision

This Application has been assessed against the requirements of section 29 of the FSANZ Act. FSANZ recommends the draft variation to Standard 1.3.3.

Decision

FSANZ approves the draft variation to the Table to clause 17 of Standard 1.3.3 – Processing Aids, to permit the use of the enzyme asparaginase sourced from *Aspergillus niger* expressing the *A. niger* asparaginase gene.

10.1 Reasons for Decision

FSANZ approves the draft variation to Standard 1.3.3 for the following reasons.

- A detailed safety assessment has concluded that the use of the enzyme does not raise any public health and safety concerns.
- Use of the enzyme is technologically justified as a treatment to reduce the formation of acrylamide in some foods.
- The impact analysis concluded that the benefits of permitting the use of the enzyme to reduce the formation of acrylamide in some treated foods outweigh any associated costs.
- The proposed variation is consistent with the FSANZ Act Section 18 objectives.

11. Implementation and Review

The FSANZ Board's decision will be notified to the Ministerial Council. Following notification, the proposed draft variation to the Code is expected to come into effect on gazettal, subject to any request from the Ministerial Council for a review of FSANZ's decision.

ATTACHMENTS

1. Draft variation to the *Australia New Zealand Food Standards Code*
2. Summary of issues raised in public submissions
3. Safety assessment report
4. Food technology report

Attachment 1

Draft variation to the *Australia New Zealand Food Standards Code*

Subsection 87(8) of the FSANZ Act provides that standards or variations to standards are legislative instruments, but are not subject to disallowance or sunsetting

To commence: on gazettal

[1] ***Standard 1.3.3*** *of the Australia New Zealand Food Standards Code is varied by inserting in the* Table to clause 17 –

Asparaginase EC 3.5.1.1	*Aspergillus niger*

Attachment 2

Summary of Issues Raised in Public Submissions

Submitter	Option	Comments
Food Technology Association of Australia	**2**	• Supports progression
Australian Food and Grocery Council	**2**	• Supports the conclusion that the use of the enzyme does not raise any public health and safety concerns, noting that FSANZ has already evaluated asparaginase derived from A. oryzae and that JECFA will be evaluating the enzyme during its meeting in 2008. • Supports FSANZ's assessment that enforcement agencies will not need to undertake analysis work to check for the presence of the enzyme in the final food. • Notes that food manufacturers may wish to make claims about reduced levels of acrylamide for treated food once the enzyme is permitted. For such claims to be substantiated enforcement agencies may need to undertake analytical assessments. AFGC further states that there are NATA accredited laboratories capable of performing such analyses for acrylamide, such as the National Measurement Institute. • Supports the current GM labelling requirements of the Code. The GM aspect of this Application relates to inserting DNA into the host organism and not the actual enzyme. The refinement process removes any traces of the production organism from the enzyme preparation so there is no novel DNA in the enzyme or left in the treated food.
New Zealand Food Safety Authority	**2**	• Satisfied that the use of the enzyme is technologically justified. • Agrees that no public health or safety concerns were identified.
Queensland Health	**2**	• Agrees there will be public health benefits in the availability of a process to reduce the formation of acrylamide in foods. • Notes this enzyme has been placed on the priority list for JECFA evaluation and expects outcomes from this to be considered. • Notes that the inactivation temperature of the enzyme will ensure no active enzymes will remain in a final product heated above 120°C but questions the case of a product not undergoing this heating process and if the presence of any active enzymes in the final product needs to be treated as a food additive and evaluated as such. Suggests consideration may need to be given to limiting foods in with the enzyme may be used. • Notes that enforcement agencies may need to analyse enzyme treated food for the presence of the enzyme, the source organism and the concentration of acrylamide formed. This will be an added cost on the enforcement agencies.

Submitter	Option	Comments
Ivan Jeray (Private)	**1**	• Opposed to approving the enzyme due to the GM aspects of the Application. • Opposes GM foods on unproven safety, economical, environmental and ethical grounds. He raises a number of opposition points to GM food in general. Stated reasons include that GM food will induce antibiotic resistance and GM food tests on animals are inadequate. • Notes that some foods are exempt from GM labelling requirements and states that GM labelling requirements are not policed. • Questions the independence of the Office of the Gene Technology Regulator. • Notes that it was not obvious in the FSANZ Notification Circular that the enzyme had GM aspects to it.

Attachment 3

Safety Assessment Report

A1003 – ASPARAGINASE AS A PROCESSING AID

Summary and Conclusion

Application A1003 seeks approval for the use of asparaginase from *Aspergillus niger* as a processing aid. The enzyme is used as a processing aid only, and any residue would be in the form of inactivated enzyme, which would be metabolised like any other protein.

The hazard assessment concluded that:

- there was no evidence of toxicity in the 90-day toxicity study or a developmental study in rats;
- the NOEL from the 90-day toxicity study was greater than 1157 mg/kg bw per day, the highest tested dose level. This is equivalent to 1038 mg TOS/kg bw/day;
- the enzyme preparation gave no evidence of any genotoxic potential in *in vitro* assays; and
- there was no evidence of any immunologically significant amino acid sequence homology with known allergens.

Based on the available evidence it is concluded that for asparaginase derived from this recombinant strain of *A. niger* no hazard has been identified. In view of the acid lability and sensitivity to proteolytic digestion following oral ingestion of asparaginase the absence of a hazard may have been reasonably anticipated. The ADI can be considered to be 'not specified'.

1. Introduction

Application A1003 seeks approval for the use of the enzyme asparaginase from *A. niger* as a processing aid. The enzyme is also known as L-asparagine amidohydrolase (EC 3.5.1.1, CAS No. 9015-68-3), and hydrolyses asparagine to aspartic acid. The tested asparaginase preparation had an enzyme activity of 34552 ASPU/g and total organic solids (TOS) of 89.7%. One asparaginase unit (ASPU) is the amount of enzyme that produces one µmole ammonia per minute under specific reaction conditions. The products of this reaction, aspartic acid and ammonia, are normal constituents of food.

The applicant's intent is for the enzyme preparation to be used as a processing aid in wheat dough-based products such as cookies and crackers, as well as other processed foods such as potato chips and French fries. Asparaginase will be inactivated during the cooking of these foods.

2. Evaluation of the safety studies

A bioinformatics analysis for homology of the asparaginase protein sequence with known protein toxins and allergens was submitted in support of this application, as were four toxicological studies. These were:

1. a 90-day sub-chronic oral toxicity study in rats;
2. a developmental toxicity study in rats;
3. a *Salmonella* / *Escherichia coli* reverse mutation assay (Ames test); and
4. a human lymphocyte assay for chromosomal aberrations.

3.1 Potential allergenicity of asparaginase

Aspergillus niger asparaginase was assessed for potential allergenicity by comparing its amino acid sequence with those of known allergens. The comparison was performed according to the FAO/WHO decision tree making use of the Structural Database of Allergenic Proteins (Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology, 2001). No immunologically significant sequence homology was detected.

3.2 Sub-chronic toxicity study

Lina, B.A.R. (2006b) Repeated-dose (13-week) oral toxicity study with an enzyme preparation of *Aspergillus niger* containing asparaginase activity (ASP72) in rats. Unpublished report No. V6998 from TNO, Zeist, The Netherlands.

In a study conducted in accordance with GLP requirements and largely to OECD test guideline 408, groups of 20 male and 20 female Wistar outbred (Crl:WI(WU) BR) rats received diets containing asparaginase (batch APE 0604, activity 34552 ASPU/g, 89.7% TOS) at a concentration of 0, 0.2, 0.6 or 1.8% (w/w) for 13 weeks. The dose selection was based on the results of an earlier two-week range-finding study in rats, where concentrations of asparaginase up to 1.8% (w/w) in the diet did not produce any adverse effects (Lina, 2006a). Since no correction for changes in rat bodyweight over the duration of the study the actual daily dose slightly declined. The average daily dose in each group was calculated to be 130, 391, and 1157 mg/kg bw/day respectively in males and 151, 452, and 1331 mg/kg bw/day respectively in females.

The experimental parameters determined were clinical signs, body weight, food consumption, neurobehavioral testing (arena testing, FOB and motor activity) ophthalmic end-points, haematological parameters, clinical chemical end-points and urinary parameters, urinalysis, gross and microscopic appearance and organ weights. Urine for urinalysis and blood for haematology and clinical chemistry were collected from 10 rats/sex/dose on day 8 and 44 of treatment and then in all rats (20/sex) during necropsy (day 91/92). Ophthalmoscopy was performed before treatment in all rats and then only in the control and high dose groups on day 85 of treatment. All other measurements were performed on day 91/92 only.

There were no treatment-related effects observed for mortality, clinical signs, body weight gain, food consumption, food conversion efficiency, neurobehavioural effects or ophthalmoscopy.

A few transient changes in measured clinical chemistry and haematology parameters which achieved statistically significance such as an elevated monocyte count in high dose males after 2 weeks and reduced basophils in both sexes of test groups after 13 weeks were considered to have no toxicological significance because they were not dose or dose-duration related. The reduced sorbitol dehydrogenase activity observed after 13 weeks in all test groups was not considered to be toxicologically significant because they were not associated with any changes in liver histopathology. In both sexes, organ weights, macroscopic pathology and histopathology were unaffected by treatment. Overall, it can be concluded that the no-observed-effect level (NOEL) is 1157 mg/kg bw per day (i.e. 1038 mg TOS/kg bw/day), the highest dose tested in this study.

3.3 Genotoxicity studies

The results of two studies of genotoxicity with asparaginase (batch: APE0604) in vitro are summarized in Table 1. The first study was in accordance with OECD Test Guideline 471 (Bacterial Reverse Mutation Test) while the second with OECD Test Guideline 473 (*In vitro* Mammalian Chromosome Aberration Test). Both studies were certified for compliance with GLP and QA.

Table 1: Genotoxicity of asparaginase in vitro

End-point	Test system	Concentration	Result	Reference
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2uvrA	62 to 5000 µg/plate, ±S9	Negative	van den Wijngaard (2006)
Chromosomal aberration	Human lymphocytes	1st experiment: 2000, 3000 or 5000 µg/ml, ±S9 2nd experiment: 3000, 4000 or 5000 µg/ml, ±S9	Negative	Usta & de Vogel (2006)

S9, 9000 × *g* supernatant from rat liver.

3.4 Developmental toxicity study

Tegelenbosch-Schouten, M.M. (2006) Oral prenatal developmental toxicity study with an enzyme preparation of *Aspergillus niger* containing asparaginase activity in rats. Unpublished report No. V7043 from TNO, Zeist, The Netherlands.

In a study conducted in accordance with GLP requirements and largely to OECD test guideline 414, groups of 25 mated Wistar outbred (Crl:WI(WU) BR) rats received diets containing asparaginase (batch APE 0604, activity 34552 ASPU/g, 89.7% TOS) at a concentration of 0, 0.2, 0.6 or 1.8% (w/w) from gestation day 0 (sperm positive smear) to 21. The dose selection was based on the results of an earlier two-week range-finding study in rats, where concentrations of asparaginase up to 1.8% (w/w) in the diet did not produce any adverse effects (Lina, 2006a). Since there was no dose correction for changes in the pregnant rat bodyweight over the duration of the treatment the actual daily dose declined from 153.2 to 84.1 mg/kg bw/day in the low-dose group; 448.5 to 238.3 mg/kg bw/day in the mid-dose group and 1349.1 to 720.7 mg/kg bw/day in the high-dose group. The mean dose achieved over the treatment period was 136, 403 and 1205 mg/kg bw/day in the low-, mid- and high-dose group respectively.

All rats were checked at least once daily for mortality and clinical signs of toxicity and body weight and food consumption were recorded every 3–4 days until day 21 of gestation. On day 21 of gestation, all rats were sacrificed and examined macroscopically. The uterus and ovaries were removed and the weight of the 'unopened' uterus, the number of corpora lutea and the number and distribution of implantation sites (classified as live fetuses or 'dead implantations') were recorded. Post-implantation losses were further classified as early or late resorptions or dead fetuses. Conception rate, pre- and post-implantation loss were recorded. At necropsy, each fetus was weighed, sexed and examined macroscopically for external findings. The condition of the placentae, the umbilical cords, the fetal membranes and fluids were examined and individual placental weights were recorded.

Approximately half of the fetuses from each litter were examined for visceral abnormalities, whilst the remainder was examined for skeletal abnormalities.

There were no deaths, no treatment-related clinical signs, no effects on litter or fetal parameters, and pathology in adults was unaffected. Similarly food consumption and bodyweight were unaffected by treatment. There was a low (and expected) spontaneous incidence of malformations that were not associated with treatment (e.g. one each in the low and mid-dose groups). This conclusion was based on the absence of a correlation with dose or effects on litter data, post-implantation loss, live and dead fetuses, resorptions, or fetal and placental weight. The NOEL in this study of embryotoxicity/teratogenicity in rats was 1205 mg/kg bw per day (i.e. 1081 mg TOS/kg bw/day), the highest dose tested in this study.

4. JECFA consideration of asparaginase

Asparaginase from *A. niger* was evaluated by the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA) at its meeting in 2008, with an ADI of 'not specified' being established.[11,12]

5. Conclusion

Following the safety assessment of asparaginase from *A. niger*, it was concluded that:

- there was no evidence of toxicity in the 90-day toxicity study or a developmental study in rats;
- the NOEL from the 90-day toxicity study was greater than 1157 mg/kg bw per day, the highest tested dose level. This is equivalent to 1038 mg TOS/kg bw/day;
- the enzyme preparation gave no evidence of any genotoxic potential in *in vitro* assays; and
- there was no evidence of any immunologically significant amino acid sequence homology with known allergens.

Based on the available evidence it is concluded that for asparaginase derived from this recombinant strain of *A. niger* no hazard has been identified. In view of the acid lability and sensitivity to proteolytic digestion following oral ingestion of asparaginase the absence of a hazard may have been reasonably anticipated. The ADI can be considered to be 'not specified'.

References:

Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology (2001). 'Evaluation of allergenicity of genetically modified foods'.

Lina, B.A.R. (2006a). 14-day range finding/feasibility study with an enzyme preparation of Aspergillus niger containing asparaginase activity (ASP72) in rats. Unpublished report No. V6998/RF from TNO, Zeist, The Netherlands.

Lina, B.A.R. (2006b). Repeated-dose (13-week) oral toxicity study with an enzyme preparation of Aspergillus niger containing asparaginase activity (ASP72) in rats. Unpublished report No. V6998 from TNO, Zeist, The Netherlands.

[11] JECFA (2008) Asparaginase from *Aspergillus niger expressed in A. niger*, at http://www.fao.org/ag/agn/jecfa-additives/specs/monograph5/additive-504-m5.pdf, Assessed on 07 August 2008

[12] JECFA (2008) Summary and Conclusions (69th meeting; 17 – 26 June 2008) http://www.who.int/ipcs/food/jecfa/summaries/summary69.pdf, Assessed on 07 August 2008

Tegelenbosch-Schouten, M.M. (2006). Oral prenatal developmental toxicity study with an enzyme preparation of Aspergillus niger containing asparaginase activity in rats. Unpublished report No. V7043 from TNO, Zeist, The Netherlands.

Usta, B. and de Vogel, N. (2006). Chromosomal aberration test with an enzyme preparation of Aspergillus niger (ASP72) in cultured human lymphocytes. Unpublished report No. V6802/14 from TNO, Zeist, The Netherlands.

van den Wijngaard, M.J.M. (2006). Bacterial reverse mutation test with enzyme preparation of Aspergillus niger (ASP72). Unpublished report No. V6805/15 from TNO, Zeist, The Netherlands.

Attachment 4

Food Technology Report

A1003 – Asparaginase from *A. niger* as a Processing Aid

Summary

DSM Food Specialties (The Netherlands) has developed an asparaginase enzyme preparation produced from a submerged, two-step fermentation of a selected genetically modified strain of *A. niger*. This commercial asparaginase preparation complies with internationally recognised specifications for the production of enzymes.

The Applicant proposed to use the asparaginase enzyme as a processing aid to reduce the formation of acrylamide when high heat is used to process starchy foods. Asparaginase converts asparagine into aspartic acid, thus preventing the asparagine, which is one of the precursors of Maillard Browning reaction, reacting with reducing sugars to form acrylamide.

Reviews on studies using different mitigating methods to reduce acrylamide show that the application of asparaginase prior to the heating step in heat processes seems to be the most promising. All the intended applications involve heating food to a temperature above 70°C. Therefore no enzyme activity is expected to remain in the product. The additional benefit of this treatment is that no further adjustment to the formulation and process is needed, therefore maintaining the sensory properties of the products. Results of trials reported by the Applicant on the efficacy of their asparaginase enzyme preparation showed a reduction in acrylamide ranging from about 35 to 100% in the final food products.

Overall, the use of asparaginase enzyme sourced from *A. niger* expressing the *A. niger* asparaginase gene as a processing aid is technologically justified to treat food products such as breads and bakery products, cereal-based and potato-based products and reaction flavours, which are subjected to high heat, to reduce the formation of acrylamide in the final products.

Introduction

DSM Food Specialties (The Netherlands) submitted an Application to FSANZ seeking to amend the Code to permit the use of the enzyme asparaginase sourced from *A. niger* expressing a gene encoding for asparaginase from *A. niger*. This enzyme, asparaginase, is to be used as a processing aid to reduce the level of free L-asparagine, an amino acid, in food during manufacturing processes. L-asparagine is one of the main precursors in the formation of acrylamide when high heat is used in the processing of certain high starch foods, e.g. potato based products such as potato chips and French fries, and wheat dough based products such as biscuits, crackers, crisp breads, tortilla chips, pretzels and bread.

Acrylamide is a suspected carcinogen that is formed by the heat-induced reaction between a reducing sugar and asparagine, which is one of the reaction pathways of Maillard reaction. The Maillard reaction is the process that gives the brown colour and tasty flavour of baked, fried and toasted foods. A summary table and a review on reducing level of acrylamide formation in cereals and cereal products was reported by Konings *et al.* (2007), who showed that, by far, the use of asparaginase seems to be the most promising method. A latest study showed that treating blanched potato chips with asparaginase enzyme solution effectively reduced 30% of acrylamide formation compared to their control treatment (Pedreschi *et al.* 2008).

Another company also submitted an Application (Application A606) to FSANZ seeking permission to use their enzyme asparaginase sourced from *A. oryzae*. The final assessment of this application was finalised by May 2008 and permission for its use gazetted in July 2008. Enzymes from both the Applicant and the other company were produced from the same microbial source but differ in their strains, *A. niger* and *A. oryzae*, respectively. Both applications are understood to share the same aim, to convert free asparagine into aspartic acid, another amino acid that does not form acrylamide. The nutritional properties are unaffected, and so are the browning and taste aspects.

At the end of 2007, the Confederation of the Food and Drink Industries of the EU (CIAA, 2007) included asparaginase in the new version (revision 11) of its Acrylamide Toolbox, a move seen to validate the efforts of companies that have developed commercial solutions using the acrylamide-reducing enzyme. Asparaginase from *A. niger* of the Applicant has been granted a generally recognized as safe (GRAS) status by FDA (filed on 11 October 2006).

Characterisation of asparaginase

Common name:	Asparaginase
IUBMB systematic name:	L-asparagine amidohydrolase
Other names:	asparaginase II; L-asparaginase, colaspase; elspar; leunase; crasnitin; α-asparaginase
Marketing name:	PreventAse™; PreventAse L™; PreventAse M™; PreventAse W™;

IUPAC/Enzyme Commission (EC) number: 3.5.1.1
Chemical Abstracts Service (CAS) number: 9015-68-3.

The International Union of Biochemistry and Molecular Biology (IUBMB) indicate that the enzyme asparaginase hydrolyses the amide in the free amino acid, L-asparagine, to the corresponding acid L-aspartate (aspartic acid) and ammonia.

$$\text{L-asparagine} + H_2O = \text{L-aspartate} + NH_3$$

The Applicant states that for all proposed applications, the action of the enzyme asparaginase takes place before the heat processing of the food.

The enzyme is inactivated at temperatures above 70^{o}C and becomes a residual protein. Thus no enzyme activity is expected to remain in the finished products because all intended applications involved heating above this temperature, including the interior of baked bread.

The Applicant verified this expectation on baked bread and yeast extract/reaction flavour samples and showed that no asparaginase activity is present in the final product.

Production of the enzyme

The Applicant states that the asparaginase enzyme is produced by a controlled submerged, two-step, fermentation process (the fermentation medium consists of glucose, yeast extract and antifoaming agent). The enzyme preparation is manufactured in accordance with Good Manufacturing Practices. Once the fermentation has been completed the production organism is killed off by incubating with sodium benzoate at pH 4.0 to 4.5 for 10 hours at 30^{o}C. The desired enzyme is separated from the microbial biomass using simple filtrations (broth filtration, followed by polish filtration and a germ reduction filtration) and then the enzyme is concentrated by ultrafiltration (UF). The enzyme preparation in liquid form is standardised and stabilised by adding glycerol to give a final enzyme activity of 2500 ASPU (asparaginase units)/ml.

The dry enzyme preparation is obtained by spray drying the UF concentrate and standardised with either maltodextrin or wheat flour to an activity of 2500 ASPU/g.

Glycerol or glycerine (INS 422) is listed in Schedule 2 of Standard 1.3.1 as a food additive approved in many processed foods to levels determined by Good Manufacturing Practice. Schedule 2 additives are also generally permitted processing aids. Sodium benzoate (INS 211) is a permitted preservative in a number of foods specified in Schedule 1 of Standard 1.3.1, in particular for preparations of food additives to maximum levels of 1000 mg/kg (0.1%). There are no specific requirements for food additives in enzyme preparations in the Code.

Allergenicity:

Given the manufacturing process and ingredients described above, no allergenic materials (list on Table to clause 4, Standard 1.2.3) are likely to be present, except when wheat flour or maltodextrin sourced from wheat is used as fillers in the dry enzyme preparation. In cases where wheat flour or maltodextrin sourced from wheat is used, it should be declared on the label of any food that has been treated with the asparaginase enzyme preparation.

Specification

The typical composition of the commercial asparaginase enzyme preparation as indicated in the Application is:

Enzyme activity	:	2500 ASPU*/g
Enzyme solids (Total Organic Solids)	:	6-12%
Ash	:	0-1%

* ASPU (Asparaginase Units). One ASPU has been defined by the Applicant as the amount of the enzyme that produces 1 micromole of ammonia from L-asparaginase per minute under specific defined conditions described for the asparaginase assay by the Applicant.

The enzyme has been shown to exhibit activities over a pH range of 4-8. The pH optimum is 4-5. The temperature optimum for the enzyme is 50^{o}C and the enzyme is inactivated at 70^{o}C.

The Application states that the enzyme preparation complies with the international specifications relevant for enzymes, which are compiled by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), in the Compendium of Food Additives Specifications (2001) and the Food Chemical Codex (2004). These specification references are both primary sources of specifications listed in clause 2 of Standard 1.3.4 – Identity and Purity.

The specification (Certificate 3) of a batch of un-standardised enzyme taken from the Application is provided below compared to the JECFA specification.

Criteria	JECFA specification	Results for asparaginase from *A. niger*
Heavy metals as Pb (ppm)	Not more than 40	Not reported
Lead (ppm)	Not more than 5	< 0.2
Arsenic (ppm)	Not more than 3	< 0.02
Cadmium (ppm)		< 0.01
Mercury (ppm)		< 0.02
Total viable counts (cfu/g)	Not more than 50,000	< 1000
Total coliforms (cfu/g)	Not more than 30	< 10
Enteropathogenic *E. coli* (/25 g)	Negative by test	Absent
Salmonella (/25 g)	Negative by test	Absent
Antibiotic activity	Negative by test	Absent by test
Production strain (/g)		Absent as claimed by the Applicant

Apart from the enzyme complex, the asparaginase preparation may contain some substances derived form the production organism and the fermentation medium, which consist of polypeptides, proteins, carbohydrates and salts.

Technological function of the enzyme

The asparaginase enzyme preparation is intended to be used in food products that contain L-asparagine and reducing sugars and are heated during food processing, to reduce the formation of acrylamide. The amino acid asparagine and reducing sugars are found in many food raw materials (such as potatoes and wheat dough based products) and are the main reactants for acrylamide formation. The asparaginase enzyme is added to the food product before the heating process to reduce the concentration of L-asparagine and therefore reduce acrylamide formation.

Subsequent heating of the processed food to temperatures above 70°C inactivates the asparaginase enzyme so that the final food does not contain the active enzyme. The nutritional properties are unaffected, and so are the browning and taste aspects.

A literature review shows the following list of carbohydrate-rich foods that are often fried, baked or grilled and for which the food industry may use this processing aid to reduce the formation of acrylamide.

Bread and bakery products

Bread is usually made from wheat flour and sometimes potato and corn flour. These flours contain high levels of L-asparagine and carbohydrates. In breads, the acrylamide forms exclusively in the crust (Koning *et al.* 2007). The application of asparaginase to bakery products presents, as suggested by some researchers, an efficient and simple way to decrease acrylamide formation (Amrein *et al.* 2007). The enzyme is added to dough during mixing or kneading. No further adjustment to formulation or process is needed, therefore maintaining the sensory properties of the product. Amrein's studies showed that there was a reduction of 50% acrylamide content in gingerbread and about 80% in hazelnut biscuits by applying a treatment of asparaginase. No asparaginase activity was detected in these baked hazelnut biscuits.

Vass *et al.* (2004) concluded that asparaginase is the most effective in reducing acrylamide in cracker products (70% reduction) compared to the other methods tried such as replacing the reducing sugars and ammonium in the baking agent. Extensive fermentation of bread and oven-baking profile were other ways of reducing acrylamide in wheat-based products.

In the long term, optimisation of agronomy and plant breeding of wheat has the potential to reduce the concentration of acrylamide in all wheat-based foods (Koning *et al.* 2007).

Other cereal-based products

Compared to breakfast cereals made from corn, oat and rice, wheat stands out from the other common grains in being a high contributor to the formation of acrylamide. One of the reasons is that wheat contains relatively high concentration of asparagine compared to other cereals (Friedmen 2003). Variety as well as harvest year have remarkable influence on the asparagine content (varies by 540%) of cereals (Claus *et al.* 2008). Claus *et al.* (2008) has compiled a summary table on studies on the mitigation of acrylamide in cereal products, which includes the impact of formulation and product composition, process technology and addition of low molecular weight additives, such as polyphenols.

Potato-based products

As opposed to wheat and cereal based products, the limiting factors for potato products are reducing sugars (Claus *et al.* 2008). The effect of reducing sugars on the development of acrylamide in potato products is well known, as are the effects of storage, variety, process control (thermal input and pre-processing) and final preparation in lowering the reducing sugar content (Foot *et al.* 2007).

Nonetheless, some studies concluded that the use of asparaginase offers potentially significant reduction in certain prefabricated potato products (Foot *et al.* 2007). Asparaginase can be applied to potato products by soaking in the enzyme solution prior to the cooking process (Pedreschi, 2008).

Reaction flavours

Reaction flavours are also called thermal process flavours, which are typically generated from reactions between reducing sugars and protein-based ingredients such as meat extracts, hydrolysed vegetable proteins (HVP) and yeast extracts. These protein sources are also rich in L-asparagine so the heating of them with reducing sugars will lead to the formation of acrylamide. There is no current information in the literature that relates to the use of asparaginase in reaction flavour production. However, the Applicant has shown in their laboratory scale trials that acrylamide formation in yeast extract-based reaction flavours can be reduced by 70% by using yeast extract treated with asparaginase.

Efficacy studies on acrylamide reduction from the Application

Table 1 is a summary table of the results of trials performed by the Applicant on the efficacy of using their asparaginase enzyme preparation, to reduce the levels of acrylamide in the final food compared to a control (and in the case of French fries also to a blank which was treated with water only).

Table 1: Summary of reductions in acrylamide formation in food treated with asparaginase enzyme preparation, taken from the DSM Application

Food Product	Acrylamide reduction (%)
French batard-crust	47
Potato bread (batard type) -crust	75
Corn bread (batard type) -crust	36
Dutch tin bread-crust	36
Bread crust	36 - 75
Crackers	87

Food Product	Acrylamide reduction (%)
Dutch honey cake (crumb)	92
Fritters (crust)	86
Donut (crust)	87
French fries	80 vs. a control 50-60 vs. a water treatment only blank
Potato-base dough product, after frying	93
Yeast extract	100
Reaction flavours	73-80

Conclusion

The use of asparaginase enzyme sourced from *Aspergillus niger* expressing the *A. niger* asparaginase gene as a processing aid is technologically justified to treat food products such as breads and bakery products, cereal-based and potato-based products and reaction flavours, which are subjected to high heat, to reduce the formation of acrylamide in the final products.

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