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

5-05
3 August 2005

INITIAL ASSESSMENT REPORT

APPLICATION A561

PHOSPHOLIPASE A_1 AS A PROCESSING AID (ENZYME)

DEADLINE FOR PUBLIC SUBMISSIONS: 6pm (Canberra time) 14 September 2005
SUBMISSIONS RECEIVED AFTER THIS DEADLINE
WILL NOT BE CONSIDERED
(See 'Invitation for Public Submissions' for details)

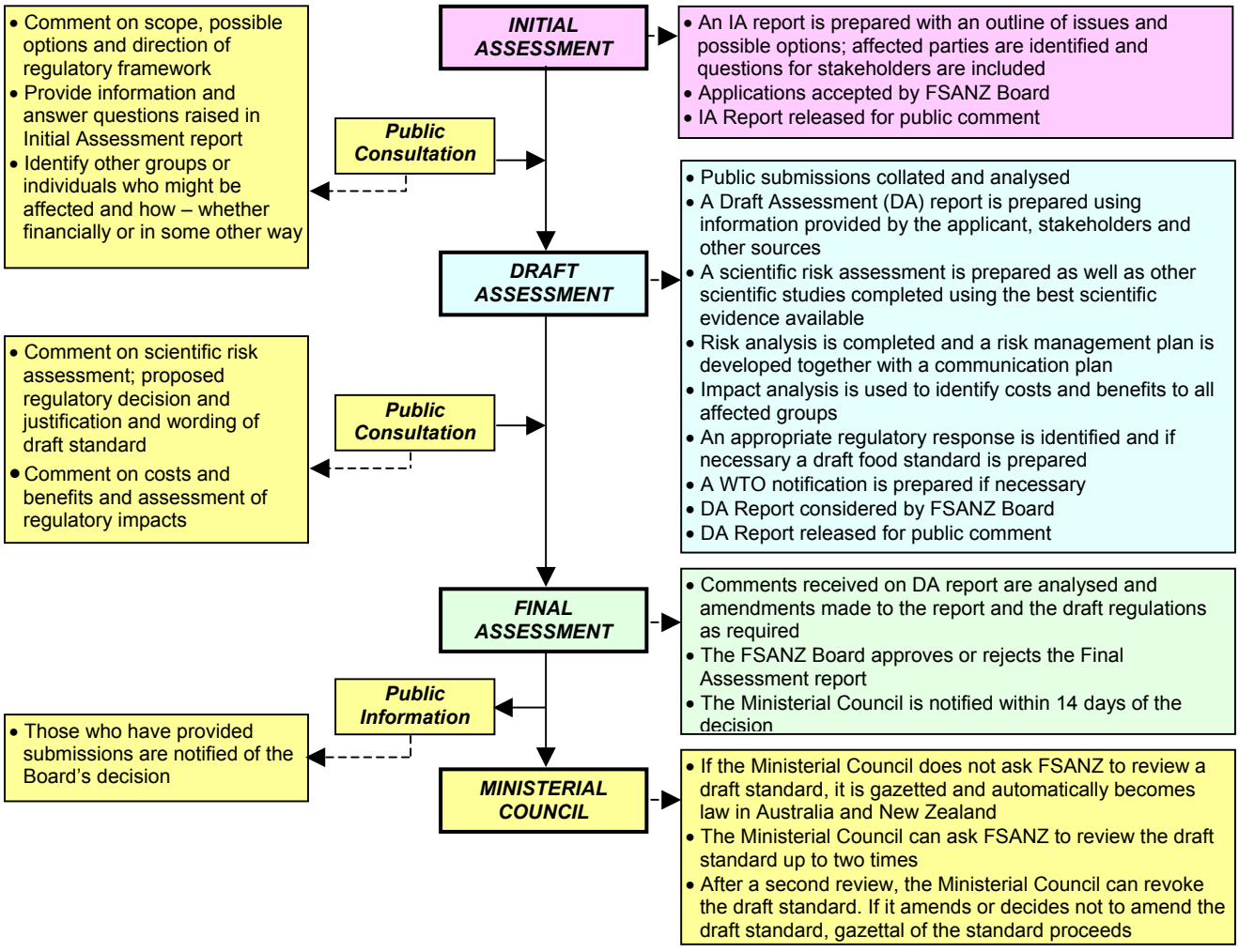
FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

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

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

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

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



INVITATION FOR PUBLIC SUBMISSIONS

FSANZ has prepared an Initial Assessment Report of Application A561, which includes the identification and discussion of the key issues.

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

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

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

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

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

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) 14 September 2005.

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

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

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.

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Executive Summary

FSANZ received an Application on 26 April 2005, from Novozymes A/S, to amend Standard 1.3.3 – Processing Aids of the *Australia New Zealand Food Standards Code* (the Code) to approve the use of a new enzyme, phospholipase A_1, as a processing aid. Phospholipase A_1 is produced, using recombinant DNA techniques, from the host *Aspergillus oryzae* containing the gene coding for phospholipase A_1 from *Fusarium venenatum*.

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

Processing aids are required to undergo a pre-market safety assessment before approval for use in Australia and New Zealand. There is currently no approval for the use of phospholipase A_1, but there is approval for phospholipase A_2. The objective of the assessment is to determine whether the Code should be amended to permit the use of phospholipase A_1 from the source *Aspergillus oryzae*, containing the gene for phospholipase A_1 isolated from *Fusarium venenatum*.

The Application states that the host organism is non-pathogenic and has a long history of safe use for food. The genetic modifications are well characterised and specific, utilising well-known plasmids so that the genetically modified *Aspergillus oryzae* is considered a safe source organism for the enzyme.

The Applicant claims phospholipase A_1 would be used in the dairy industry for cheese manufacture to improve process efficiencies and cheese yields. The enzyme acts on phospholipids to form a lysophospholipid and a free fatty acid. These reaction products have improved emulsifying properties and is claimed to produce an approximate 2% increase in cheese yield.

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

Phospholipase A_1 is already approved in Argentina, Germany, Great Britain, Italy, Sweden, Ireland, Egypt, Iran and Turkey. It has been self-affirmed as a Generally Recognized As Safe (GRAS) notification to the US Food and Drug Administration (FDA), GRAS notification GRN 000142 (FDA response letter June 2004). As well it has been submitted in Denmark and will be submitted in France in the near future.

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

- The Application seeks approval for a new enzyme from a microbial source as a processing aid.

- Microbial enzymes and their sources are listed in the Table to clause 17 of Standard 1.3.3 of the Code. There is currently no approval for phospholipase A_1 from the source *Aspergillus oryzae*, containing the gene for phospholipase A_1 isolated from *Fusarium venenatum* in this Table.

- The Application relates to a matter that warrants a variation to Standard 1.3.3, if further assessment supports such a variation.

- This Application is not so similar to any previous application that it ought not be accepted.

- There are no other regulatory measures, than a variation to the Code available to permit the use of this processing aid.

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

1. Introduction

FSANZ received an Application on 26 April 2005, from Novozymes A/S, to amend Standard 1.3.3 – Processing Aids of the Code to approve the use of a new enzyme, phospholipase A_1, as a processing aid. Phospholipase A_1 is produced, using recombinant DNA techniques, from the host microorganism *Aspergillus oryzae* containing the gene coding for phospholipase A_1 from the fungus *Fusarium venenatum*.

The Applicant claims that this new enzyme would be used in the dairy industry for cheese manufacture to improve process efficiencies and cheese yields. The phospholipase A_1 enzyme preparation catalyses the hydrolysis of diacylphospholipids to form a 2-acyl-1-lysophospholipid and a free fatty acid. The modified phospholipids from the milk are claimed to have improved emulsifying properties to keep more of the milk components in cheese and reduce losses into the waste whey stream.

2. Regulatory Problem

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

The Table to clause 17 of Standard 1.3.3 contains a list of permitted enzymes of microbial origin. There is currently no approval for the use of phospholipase A_1 as a food enzyme in the Code. Phospholipase A_2 has recently been approved as a permitted enzyme of microbial origin and is listed in the Table to clause 17 (Application A501, gazetted in the Code on 16 December 2004).

3. Objective

The objective of this assessment is to determine whether it is appropriate to amend the Code to permit the use of phospholipase A_1 from *A. oryzae* containing the gene coding for phospholipase A_1 from *F. venenatum.*

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

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

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

- the need for standards to be based on risk analysis using the best available scientific evidence;

- the promotion of consistency between domestic and international food standards;
- the desirability of an efficient and internationally competitive food industry;
- the promotion of fair trading in food; and
- any written policy guidelines formulated by the Ministerial Council.

4. Background

4.1 Historical Background

The Applicant claims that phospholipase A_1 is found naturally in animal and plant tissues. Phospholipase A_1 is widely distributed in nature and in mammals. The major sources are found in the pancreas and the brain[1]. The enzyme selectively acts on the fatty acid in position 1 (sn-1) in phospholipids to cleave a free fatty acid and form a lysophospholipid. The enzyme, and reaction by-products of enzyme use, fatty acids and lysophospholipids, are claimed to be natural components of food and as such have a history of safe use, and are no different to other constituents in food.

Phospholipase A_2 (EC number [3.1.1.4]) is currently approved as an enzyme. It is listed in the Table to clause 15 – Permitted enzymes of animal origin, being sourced from porcine pancreas. It has more recently been approved in the Table to clause 17 – Permitted enzymes of microbial origin, being sourced from *Streptomyces violaceoruber.* Phospholipase A_2 is used to hydrolyse lecithin to produce a modified lecithin which has improved emulsifying properties, especially for aqueous systems.

4.2 Work Plan Classification

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

5. Relevant Issues

5.1 Nature and technological justification of the enzyme

The common name for the enzyme is phospholipase A_1. The systematic name for the enzyme is phosphatidylcholine 1-acylhydrolase[2]. The phospholipase A_1 enzyme has the Enzyme Commission (EC) number of [3.1.1.32] and the Chemical Abstracts Service (CAS) Registry Number of 9043-29-2. The molecular weight of the enzyme is listed by the Applicant as 110 -115 kDa. The enzyme preparation is a clear pale yellow liquid which is water soluble.

The phospholipase A_1 catalyses the reaction of:

[1] Encyclopedia of Food Sciences and Nutrition, Phospholipids, Second Edition, Academic Press, (2003), 4528-4529.
[2] International Union of Biochemistry and Molecular Biology (IUBMB) Enzyme Nomenclature http://www.chem.qmul.ac.uk/iubmb/enzyme/EC3/1/1/32.html, accessed on 5 May 2005.

phosphatidylcholine +H_2O = 2-acylglycerophosphocholine + carboxylate (fatty acid).

This reaction is comparable to that which the enzyme phospholipase A_2 catalyses being:

phosphatidylcholine +H_2O = 1-acylglycerophosphocholine + carboxylate (fatty acid).

Phospholipase A_1 attacks and cleaves the fatty acid from the number 1 position (sn-1) of the glycerol backbone of lecithin (so leaving the acyl group remaining on the number 2 position, hence the name of the reaction product being 2-acyl), while phospholipase A_2 attacks the number 2 position (sn-2). Phospholipase A_1 is stated to have much broader specificity than phospholipase A_2[1].

The Applicant claims that the enzyme preparation is used to improve process efficiencies in cheese manufacture with lower losses of fat and other solids into the whey stream. The phospholipase A_1 enzyme preparation is added to the milk used for cheese manufacture before the coagulant is added. The phospholipids produced after the enzyme treatment have better emulsifying properties to untreated milk and as such keep more of the milk components in the cheese and less lost in the whey stream. The Applicant claims the cheese yields are increased by approximately 2.0%, without a change to the quality or composition of the cheese.

The phospholipase A_1 enzyme preparation is produced by submerged fermentation of the microbial source *A. oryzae* that has the gene coding for phospholipase from *F. venenatum* inserted by recombinant DNA techniques.

It is unlikely that there are any dietary or nutrition implications with this Application. The enzyme is to be used as a processing aid and the majority of the enzyme will be removed from the final product as part of the process. Some small proportion of the enzyme may remain in the final product (cheese) but it has no technological function once there is no substrate to act on. Any remaining substrate will be unavailable to react with the enzyme since it will be bound in the resultant solid cheese matrix. Enzymes and their reaction by-products, lysophospholipids and fatty acids are natural components of food and no different to other constituents of food.

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

5.2 Safety assessment

The host microorganism *A. oryzae*, is stated by the Applicant to be non-pathogenic and has a long history of safe use in food. It is also the source organism for a number of approved enzymes in the Table to clause 17 of Standard 1.3.3. *A. oryzae* has also been used as the host microorganism for a number of other approved source microorganisms produced using recombinant DNA techniques, which are the sources for approved enzymes in the Table to clause 17 of Standard 1.3.3.

The fungus *F. venenatum* has not been used as a donor organism for any approved enzyme sources in this Table or within the Code. However the micro-organism *F. venenatum* is the donor organism for a GM source of a xylanase enzyme (xylanase derived from *F. venenatum* carrying a gene encoding xylanase from *Thermomyces lanuginosus*) which has a USA Food and Drug Administration (FDA) GRAS notice of GRN 000054.

This enzyme and source was also evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in their recent Technical Report in 2004[3] (an ADI of 'not specified' was allocated and a specification prepared). *F. venenatum* is also the fungal source of the myco-protein used to produce products for food consumption[4], since 1985 under the trade name 'Quorn'[5]. In this case the myco-protein is a meat (chicken or beef) substitute[5].

The Applicant believes the genetic modifications to produce the enzyme source are well characterised and specific, utilising well-known plasmids for the vector constructs, and because the introduced genetic material does not encode and express any known harmful or toxic substances, it is considered a safe source.

The Applicant has provided the following studies:

- 13 weeks sub-chronic oral toxicity study in rats
- Test for mutagenic activity (Ames test)
- Human lymphocyte cytogenetic assay.

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

5.3 Other international regulatory standards

The Applicant states that the enzyme can already be legally sold in Argentina, Germany, Great Britain, Italy, Sweden, Ireland, Egypt, Iran and Turkey.

The same enzyme from the same Applicant has recently been deemed self-affirmed GRAS in the USA. A letter of no objection dated June 23 2004 for this enzyme is the GRAS notice No. GRN 000142.

As well it has been submitted in Denmark and will be submitted in France in the near future.

The Applicant claims the phospholipase A_1 enzyme preparation complies with the specifications for enzyme preparations in the Food Chemicals Codex, 5th Edition, 2004 and JECFA Compendium of Food Additive Specifications, Volume 1, Annex 1, Addendum 9 2001, (General Specifications and Considerations for Enzyme Preparations Used in Food Processing). The enzyme preparation is also claimed to comply with the proposed guidelines of the Scientific Committee on Food (SCF) of the European Union for food enzyme preparations.

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[3] World Health Organization Technical Report Series, 2004;922:1-176, Evaluation of certain food additives and contaminants.
http://whqlibdoc.who.int/trs/WHO_TRS_922.pdf, accessed on 20 May 2005.

[4] Wiebe, M.G. (2002) Myco-protein form *Fusarium venenatum*: a well established product for human consumption. Appl. Microbiol. Biotechnol. **58**(4):421-427

[5] Berka, R.M., Nelson, B.A., Zaretsky, E.J., Yoder, W.T. and Rey, M.W. (2003) Genomics of Fusarium venenatum: an alternative fungal host for making enzymes. In: Arora, D.K. and Khachatourians, G.G., eds. *Applied Mycology & Biotechnology, Vol.4, Fungal Genomics*, Elsevier Science, Amsterdam.

6. Regulatory Options

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

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

Option 1. **Not approve** the use of phospholipase A1 from *Aspergillus oryzae* containing the gene coding for phospholipase A1 from *Fusarium venenatum* as a processing aid.

Option 2. **Approve** phospholipase A1 from *Aspergillus oryzae* containing the gene coding for phospholipase A1 from *Fusarium venenatum* as a processing aid.

7. Impact Analysis

7.1 Affected Parties

The affected parties to this Application include the following:

1. those sectors of the food industry wishing to produce and market food products produced using this enzyme, specifically cheese manufacturers;

2. consumers; and

3. Australian, State, Territory and New Zealand Government agencies that enforce food regulations.

7.2 Impact Analysis

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

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

8. Consultation

8.1 Public consultation

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

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

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

8.2 World Trade Organization (WTO)

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

Amending the Code to approve the enzyme phospholipase A_1 from *A. oryzae* containing the gene coding for phospholipase A_1 from *F. venenatum* as a processing aid is unlikely to have a significant effect on trade. The enzyme preparation is consistent with the international specifications for food enzymes of Food Chemicals Codex (5th Edition, 2004) and JEFCA so there does not appear to be a need to notify the WTO. This issue will be fully considered at Draft Assessment and, if necessary, notification will be recommended to the agencies responsible in accordance with Australia's and New Zealand's obligations under the WTO Technical Barrier to Trade (TBT) or Sanitary and Phytosanitary Measure (SPS) Agreements. This will enable other WTO member countries to comment on proposed changes to standards where they may have a significant impact on them.

9. Conclusion and Recommendation

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

- The Application seeks approval for a new enzyme from a microbial source as a processing aid.

- Microbial enzymes and their sources are listed in the Table to clause 17 of Standard 1.3.3 of the Code. There is currently no approval for phospholipase A_1 from the source *A. oryzae*, containing the gene for phospholipase A_1 isolated from *F. venenatum* in this Table.

- The Application relates to a matter that warrants a variation to Standard 1.3.3, if further assessment supports such a variation.

- This Application is not so similar to any previous application that it ought not be accepted.

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