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

6 July 2011
[13-11]

APPLICATION A1050 GLYCEROPHOSPHOLIPID CHOLESTEROL ACYLTRANSFERASE AS A PROCESSING AID APPROVAL REPORT

Executive Summary

Food Standards Australia New Zealand (FSANZ) received an Application from Danisco A/S via Axiome Pty Ltd on 29 June 2010. The Application is seeking approval to permit a protein engineered glycerophospholipid cholesterol acyltransferase derived from a genetically modified (GM) *Bacillus licheniformis,* expressing an acyltransferase encoding gene sequence from *Aeromonas salmonicida* subsp. *salmonicida*. The commercial name for the enzyme preparation is KLM3′. However, for brevity, this Report will refer to the enzyme as acyltransferase.

The Application was assessed under the General Procedure with one round of public consultation.

The specific objectives in considering this Application were to:

- protect public health and safety in relation to use of the enzyme processing aid in a range of foods

- ensure adequate information relating to the enzyme processing aid is provided to consumers to enable informed choice

Acyltransferase (EC 2.3.1.43) is an enzyme that transfers acyl[1] groups from phospholipids and glycolipids to acceptors such as sterols (i.e. cholesterol and plant sterols), fatty alcohols and other smaller primary alcohols. It also exhibits the enzymatic activities of phospholipase (EC 3.1.1.4) and lysophospholipase (EC 3.1.1.5). The proposed use of acyltransferase is as a processing aid to improve emulsification in:

- egg yolk and whole eggs to avoid product separation during high temperature processing in the manufacture of mayonnaise
- processed meat products to improve the emulsification of fat in the product which improves consistency and reduces cooking loss
- degumming of vegetable oils
- production of UHT and powdered milk to reduce fouling

[1] An organic radical having the general formula RCO, derived from the removal of a hydroxyl group from within the carboxyl group of an organic acid

- yoghurt to facilitate fermentation and improve viscosity
- bakery products containing eggs to give a softer and more tender crumb

A pre-market assessment of the safety of the enzyme, including the source and donor organisms, as well as assessment of the technological suitability, is required prior to any approval being granted. Processing aids used in food manufacture are regulated under Standard 1.3.3. No permissions currently exist for acyltransferase from any source.

To date, there has been no evaluation of acyltransferase from genetically modified (GM) *B. licheniformis* by the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA). A 'no-objections' letter was received by the Applicant in response to an independent GRAS (generally recognized as safe) assessment (GRAS Notice 265) for the enzyme in the United States and it is currently under review in Brazil.

The acyltransferase enzyme preparation complies with relevant international specifications for enzyme preparations prepared by JECFA (2006) and specifications of the Food Chemicals Codex (FCC), 6th Ed, 2008.

Risk Assessment

The risk assessment considered the technological suitability, the potential hazard and identity of the donor and host microorganisms, as well as assessing the potential hazard of the acyltransferase enzyme preparation. The impact of any changes to the lipid composition of the final food products as a result of the use of the enzyme was also considered including whether such changes could have a negative effect on the blood lipid profile of consumers.

Key findings of the evaluation are:

- *B. licheniformis* as the host organism is a well-characterised expression system for the production of enzymes and has a long history of safe use.

- There was no evidence of toxicity at the highest dose tested in a 90-day repeat dose study. The No Observed Adverse Effect Level (NOAEL) was 41 mg/kg bw/day, the highest dose tested.

- There was also no evidence of genotoxicity.

- Based on the reviewed toxicological data it was concluded that in the absence of any identifiable hazard, an ADI (Acceptable Daily Intake) does not need to be specified.

- Based on the available evidence, acyltransferase produced in a GM *B. licheniformis* is considered safe for use in foods for human consumption.

- The stated purpose for this acyltransferase is to improve the emulsification properties of various foods. When used in the form and amounts prescribed, the enzyme is technologically justified and achieves its stated purpose.

- There is no negative impact on the lipid composition of foods produced using the enzyme.

- The enzyme meets international purity specifications.

Labelling

Standard 1.5.2 – Food produced using Gene Technology, outlines provisions for labelling of foods produced using gene technology. Although processing aids are not normally subject to labelling on the final food, under paragraph 4(1)(d) of Standard 1.5.2, labelling requirements do apply where novel DNA and/or novel protein from the processing aid remains present in the final food.

It would be the responsibility of a food manufacturer using acyltransferase to determine if there is residual enzyme or fermentation products in the final food. If novel protein were to remain in the final food, food produced using acyltransferase would be required to be labelled 'genetically modified' in conjunction with the name of the processing aid. Labelling provisions of Standard 1.2.3 – Mandatory Warning and Advisory Statements and Declarations, for the declaration of gluten and soybean would also apply should residual amounts of fermentation nutrients, present in the enzyme preparation, be carried over to the final food.

An analytical method to assay acyltransferase in fermentation broths, concentrates and formulated products was provided by the Applicant.

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 a food regulatory measure developed or varied as a result of the Application outweigh the direct and indirect benefits to the community, Government or industry that would arise from the development or variation of the food regulatory measure

- whether other measures (available to the Authority or not) would be more cost-effective than a variation to Standard which could achieve the same end

- any relevant New Zealand standards

- any other relevant matters.

Decision

To approve a variation to Standard 1.3.3, subject to amendment, to permit the use of a protein-engineered variant of acyltransferase produced by a genetically modified *Bacillus licheniformis* as a processing aid.

Reasons for Preferred Approach

An amendment to the Code approving the use of the acyltransferase enzyme preparation as a processing aid in Australia and New Zealand is proposed on the basis of the available evidence 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.

- The source organism, *B. licheniformis,* has an established safe history of use in the production of food enzymes.

- Use of acyltransferase as a processing aid is technologically justified and would be expected to provide benefits to food manufacturers in terms of product quality, yield and manufacturing processes. Potential benefits may also exist for consumers in the provision of products with improved and consistent quality.

- Permitting use of the enzyme would not impose significant costs for government agencies, consumers or manufacturers.

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

- There are no relevant New Zealand standards.

Consultation

As this Application was assessed under the General Procedure, there was one round of public comment. Public submissions were invited on the Assessment Report between 4 February 2011 and 18 March 2011. Comments were specifically requested on the scientific aspects of the Application, including the technological function and any information relevant to the safety assessment of the enzyme acyltransferase produced by a genetically modified strain of *B. licheniformis* to be used as a processing aid.

A total of two submissions were received as a result of the public consultation. These are summarised in **Attachment 3** and were considered in developing this Approval Report.

Amendments to Draft Variations after Consultation

In response to a submission on the Assessment Report, the name of the enzyme has been amended to 'glycerophospholipid cholesterol acyltransferase' which better and more fully describes the enzyme than the name previously advised. The revised term also aligns with that used in the USFDA GRAS listing and in the Codex Committee on Food Additives (CCFA) Inventory of Processing Aids (IPA). Retention of the previous name, which more accurately referred to a group of enzymes rather than to this specific enzyme, may have resulted in some confusion if a future application was received for another enzyme belonging to this group. The amended enzyme name has also been reflected in records and reports related to this Application.

CONTENTS

SUPPORTING DOCUMENT

The following material, which was used in the preparation of this Approval Report, is available on the FSANZ website at:

http://www.foodstandards.gov.au/foodstandards/applications/applicationa1050acyl4901.cfm

SD1: Risk Assessment Report (Approval)

Introduction

Food Standards Australia New Zealand (FSANZ) received an Application from Danisco A/S via Axiome Pty Ltd on 29 June 2010. The Application seeks approval for the use of glycerophospholipid cholesterol acyltransferase derived from a genetically modified (GM) *Bacillus licheniformis,* expressing an acyltransferase encoding gene sequence from *Aeromonas salmonicida* subsp. *salmonicida* as a processing aid. The commercial name for the enzyme is KLM3′. For brevity, this report will refer to the enzyme as acyltransferase where appropriate.

The proposed use of acyltransferase is as a processing aid to improve emulsification in a range of foods and food manufacturing processes. The Applicant claims acyltransferase could replace or partially replace phospholipase and other emulsification agents currently used in:

- egg yolk and whole eggs to modify phospholipids to lysophospholipids and cholesterol-esters in egg yolk which in turn avoids product separation at high temperature pasteurisation during production of mayonnaise
- processed meat products to improve emulsification which contributes to improved consistency and reduced cooking loss
- degumming of vegetable oils
- production of UHT and powdered milk to reduce fouling
- yoghurt to facilitate improved fermentation and viscosity
- bakery products containing eggs to give a softer and more tender crumb

1. The Issue / Problem

The Applicant proposes the use of a protein engineered acyltransferase as a processing aid to replace or partially replace phospholipase and other emulsification agents in various food applications.

A pre-market assessment and approval is required before any new processing aid is permitted. Consideration of a safety assessment of the enzyme, including the source and donor organisms, as well as assessing the technological function of the enzyme for its claimed use is required, before any permission may be granted.

2. Current Standard

2.1 Current Standard

Processing aids used in food manufacture are regulated under Standard 1.3.3. In clause 1 of the Standard, a processing aid is described as:

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 Table to clause 17 – Permitted enzymes of microbial origin, contains a list of permitted enzymes and the microorganisms (including genetically modified organisms) from which they can be produced. Currently, no permission exists in the Code for acyltransferase from either genetically modified or non-genetically modified sources.

2.2 International regulations

This acyltransferase enzyme was subject to an independently assessed GRAS (generally recognized as safe) determination (GRN: 265) in the United States, with a 'no-objection letter' issued by the United States Food and Drug Administration (USFDA). It is currently under review in Brazil.

The enzyme has not been evaluated by the FAO/WHO Joint Expert Committee on Food Additives (JECFA), however identity and purity specifications written for the acyltransferase enzyme preparation do comply with the relevant international specifications prepared by JECFA (2006) and specifications of the Food Chemicals Codex, 6th Ed, 2008.

2.3 Nature of the Enzyme and Source of Organism

Acyltransferase (EC 2.3.1.43) transfers acyl groups from phospholipids and glycolipids to acceptors such as sterols, fatty alcohols and other smaller primary alcohols. It also exhibits the enzymatic activity of phospholipase (EC 3.1.1.4) and lysophospholipase (EC 3.1.1.5).

The source organism used to produce this acyltransferase is a GM *B. licheniformis* with a history of safe use in the production of food enzymes. The modified *B. licheniformis* expresses a codon-optimised gene for a protein engineered variant of acyltransferase produced from *Aeromonas salmonicida* subsp. *salmonicida. Aeromonas salmonicida* subsp *salmonicida* is classified as biosafety level 1 by ATCC (not known to cause disease in healthy adult humans).

2.4 Technological purpose

The proposed use of acyltransferase is as a processing aid to improve emulsification. Improved emulsification results from the enzyme's effect on phospholipids and glycolipids present in the cell membranes of certain foods. Proposed uses are in:

- egg yolk
- mayonnaise and cakes containing whole eggs
- degumming of oil
- processed meats
- UHT and powdered milk
- yoghurt

The enzyme's effectiveness in improving emulsification is based on the effect the enzyme has on the cell membrane by transferring acyl groups from phospholipids to acceptors such as sterols. The hydrolysis reaction leads to the release of less hydrophobic and thus more water-soluble lysophospholipids, which have a higher dynamic surface activity in the aqueous phase. Lysophospholipids are excellent emulsifiers and oil-in-water emulsions stabilised by hydrolysed phospholipids show improved heat stability.

This acyltransferase predominantly hydrolyses the following reaction:

Phosphatidylcholine + cholesterol → 1-acylglycerophosphocholine + a cholesterol ester

It transfers the fatty acid moiety (palmitoyl, oleoyl or linoleoyl) from the *sn*-2 position in phosphatidylcholine to cholesterol.

In addition to the above reaction, acyltransferase also exhibits the enzymatic activities of phospholipase (EC 3.1.1.4) and lysophospholipase (EC 3.1.1.5). Phospholipase hydrolyses the ester bond in the *sn*-2 position of phosphatidylcholine to release a free fatty acid, while lysophospholipase performs the reverse reaction; esterification of a free fatty acid to the *sn*-2 position of lysophosphatidylcholine.

The Applicant states impurity and microbial specifications written for the enzyme meet specifications laid down by the FAO/WHO Expert Committee on Food Additives (JECFA, 2006) and the Food Chemicals Codex (FCC, 2008). These monographs are primary reference sources listed in Clause 2 of Standard 1.3.4 – Identity and Purity. Based on the provided information, FSANZ agrees that acyltransferase produced from a genetically modified strain of *B. licheniformis* meets international specifications for enzyme preparations.

3. Objectives

The objective of this Assessment is to determine whether it is appropriate to amend Standard 1.3.3 to permit the use of the engineered acyltransferase enzyme from a genetically modified *B. licheniformis* strain for use as a processing aid.

In developing or varying a food standard, FSANZ is required by its legislation to meet three primary objectives which are set out in section 18 of the FSANZ Act. These are:

- the protection of public health and safety; and
- 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.

The Ministerial Council Policy Guideline: *Addition to Food of Substances other than Vitamins and Minerals* includes policy principles in regard to substances added to achieve a solely technological function such as food additives and processing aids. According to these guidelines, permissions should be granted where:

- the purpose for adding the substance can be articulated clearly by the manufacturer as achieving a solely technological function (i.e. the ‘stated purpose’);
- the addition of the substance to food is safe for human consumption;

- the amounts added are consistent with achieving the technological function;

- the substance is added in a quantity and a form which is consistent with delivering the stated purpose; and

- no nutrition, health or related claims are to be made in regard to the substance.

4. Questions to be answered

The primary objective of most relevance to the assessment of this Application is the protection of public health and safety. In order to specifically address this, FSANZ has performed a risk assessment to determine if there are any public health and safety concerns associated with the proposed use.

The risk assessment has been based on the best available scientific evidence and considers the following questions:

- Does the enzyme preparation present any food safety issues?
- Does the enzyme achieve its stated technological purpose?

RISK ASSESSMENT

A detailed assessment of the safety and functionality of acyltransferase has been undertaken for this Application. A summary of the assessment (Supporting Document 1) is presented below.

In addition to information supplied by the Applicant, other available resource material including published scientific literature and general technical information was used in this assessment.

5. Risk Assessment Summary

The risk assessment has considered the technological suitability, the potential hazard and identity of the donor and host microorganisms, as well as assessing the safety of the acyltransferase preparation. The impact of any changes to the lipid composition of the final food products as a result of the use of the enzyme was also considered including whether such changes could have a negative effect on the blood lipid profile of consumers.

Based on the available data, no food safety concerns have been identified with the enzyme, or with the donor or host organisms used to produce the enzyme, which would preclude permitting its use as a food processing aid. The absence of any specific hazards being identified is consistent with the enzyme undergoing normal proteolytic digestion in the gastrointestinal tract.

It was further concluded that the Application clearly articulates the stated purpose for this acyltransferase, namely to improve emulsification in the proposed foods. The evidence submitted in support of the Application provides adequate assurance that the enzyme, in the form and amounts added, is technologically justified and has been demonstrated to be effective in achieving its stated purpose. Further, there will be no negative impact on the lipid composition of foods produced using this enzyme.

The available data are considered sufficient to provide an acceptable level of confidence in the conclusions of this risk assessment in regard to the safety and suitability of this enzyme for its stated purpose.

5.1 Conclusions

- *B. licheniformis* as the host organism is a well-characterised expression system for the production of enzymes, and has a long history of safe use.

- There was no evidence of acyltransferase toxicity at the highest dose tested in a 90-day repeat dose study. The No Observed Adverse Effect Level (NOAEL) was 41 mg/kg bw/day, the highest dose tested.

- There was also no evidence of genotoxicity.

- Based on the reviewed toxicological data, it was concluded that in the absence of any identifiable hazard, an Acceptable Daily Intake (ADI) does not need to be specified.

- Based on the available evidence, acyltransferase produced in *B. licheniformis* is considered safe for use in foods for human consumption.

- The stated purpose for this acyltransferase is to improve the emulsification properties of various foods. When used in the form and amounts prescribed, the enzyme is technologically justified and achieves its stated purpose.

- There is no negative impact on the lipid composition of foods produced using the enzyme.

- The enzyme meets international purity specifications.

Risk Management

6. Risk Management Measures

6.1 Addressing the objectives

The legislative objectives that FSANZ is required to meet when developing or varying a food standard are noted in section 3. FSANZ considers the primary objective of most relevance to this Application is the protection of public health and safety. The other two have less direct relevance although are also taken into consideration.

6.1.1 Risk to public health and safety

FSANZ concludes that approval for use of a protein engineered acyltransferase sourced from genetically modified *B. licheniformis* as a processing aid does not pose a public health and safety risk to Australian and New Zealand consumers.

6.1.2 Providing adequate information to enable informed choice – Labelling

Labelling addresses the objective set out in section 18(1)(b) of the FSANZ Act; the provision of adequate information relating to food to enable consumers to make informed choices.

Although processing aids are not normally subject to labelling on the final food, under clause 4(1)(d) of Standard 1.5.2, labelling requirements do apply for processing aids where novel DNA and/or novel protein from the processing aid remains present in the final food. It would be the responsibility of a food manufacturer using acyltransferase to determine if there is residual enzyme or fermentation products in the final food.

If novel protein were to remain in the final food, food produced using acyltransferase would be required to be labelled 'genetically modified' in conjunction with the name of the processing aid. Additionally, labelling provisions of Standard 1.2.3 would also apply should certain residual fermentation nutrients present in the enzyme preparation be present in the final food.

FSANZ considers the current labelling provisions included in the Code are appropriate and no other mandatory labelling is necessary.

6.1.3 Prevention of misleading and deceptive conduct

FSANZ has considered this objective and concludes there are no misleading or deceptive conduct aspects to this assessment.

6.1.4 Consistency with Policy Guidelines

As noted in section 3, FSANZ is required to have regard to the Policy Guideline on the Addition to Foods of Substances other than Vitamins and Minerals. Since the purpose for use of the acyltransferase enzyme in food falls under 'Technological Function', regard has been given particularly to the specific order policy principles for 'Technological Function'.

It has been determined that the Applicant provided a clear stated purpose; use of acyltransferase as a processing aid is safe; there is a clear technological function and the enzyme is added in a quantity and form which is consistent with achieving the stated purpose.

7. Options

As processing aids require a pre-market approval under Standard 1.3.3, it is not appropriate to consider non-regulatory options. Consequently, two regulatory options have been identified for this Application:

Option 1: Reject the Application

Option 2: To approve a draft variation to amend Standard 1.3.3 to permit the use of acyltransferase produced by a genetically modified *B. licheniformis* as a processing aid

8. Impact Analysis

FSANZ is required to consider the impact of various regulatory and non-regulatory options on all sectors 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.

In accordance with the Best Practice Regulation Guidelines, completion of a preliminary assessment for this application indicated a low or negligible impact. The Office of Best Practice Regulation has advised that the application appears to be of a minor or machinery nature; notified approval of the preliminary assessment (RIS ID: 11818) and further advised that a Regulatory Impact Statement (RIS) is not required.

8.1 Affected Parties

The affected parties may include:

- the enzyme manufacturer and those sectors of the food industry wishing to produce and market foods manufactured using this acyltransferase enzyme as a processing aid
- consumers of food products in which acyltransferase is used as a processing aid
- government agencies with responsibility for compliance and enforcement of the Code.

8.2 Benefit Cost Analysis

8.2.1 Option 1: Reject the Application

This option is the *status quo*, with no changes required to the Code.

If rejected, food industries and, potentially, consumers, may be disadvantaged as they would be unable to capture the benefits conferred by the technological function of the new enzyme.

8.2.2 Option 2: Approve the draft variation

This option allows the food industry choice in relation to the type of enzyme available for use in their food product. For the proposed foods, the Applicant claims acyltransferase improves emulsification properties, including emulsion stability under heat, and would provide the following product and processing benefits:

- Reduced product separation during high temperature processing in the manufacture of egg yolk/whole egg mayonnaise
- Improved emulsification which improves consistency and reduces cooking loss in processed meat products
- Increased yields during degumming of vegetable oils
- Reducing fouling in production of UHT and powdered milk
- Improvements in fermentation and viscosity during yoghurt manufacture
- Softer and more tender crumb in bakery products containing eggs

Approving the Application would allow manufacturers of foods produced using this enzyme to benefit from the identified improvements in product quality, yield and manufacturing processes. Improvements in the quality of products manufactured using this acyltransferase may provide potential benefit to consumers.

Use of processing aids by manufacturers is voluntary and not subject to limits in the Code. Therefore, there is not predicted to be any significant cost impost on jurisdictions to determine compliance with the proposed amendment compared with current monitoring and compliance activities. Similarly, there should be no additional costs imposed on consumers.

8.3 Comparison of Options

Option 1 does not appear to impart any apparent benefit to industry, consumers or government while denying industry access to a safe and technologically justified processing aid.

Option 2 does not appear to impose any significant costs on industry, consumers or government. It provides benefits to industry in terms of product quality, yield and manufacturing processes. Potential benefits may exist for consumers in the provision of products with consistent high quality.

In considering the costs and benefits associated with both options, Option 2 would be preferred as it conveys benefits to the food industry and potential benefits to consumers without imposing significant costs for government agencies, consumers or manufacturers.

Communication and Consultation Strategy

9. Communication

FSANZ developed and applied a basic communication strategy to this Application. The strategy involved notifying interested parties and email alert subscribers to the availability of the assessment reports for public comment and placing the reports on the FSANZ website.

The process by which FSANZ considers standards matters is open, accountable, consultative and transparent. The purpose of inviting public submissions is to obtain the views of interested parties on the issues raised by the application and the impacts of regulatory options. The issues raised in the public submissions are evaluated and addressed in FSANZ assessment reports.

The Applicant, individuals and organisations making submissions on this Application are notified at each stage of the Application. The decision of the FSANZ Board to approve the variation to the Code will be notified to the Ministerial Council. If a request to review the decision is not made by the Ministerial Council, the variation will be gazetted. Stakeholders (including the Applicant) and submitters will be advised of the notification and gazettal directly and via the FSANZ website.

10. Consultation

10.1 Public Consultation

The Assessment Report was notified for public comment between 4 February 2011 and 18 March 2011. As this Application was assessed under a General Procedure, only one round of public comment was applicable.

Comments were sought in relation to scientific aspects of the Application including the technological function and any information relevant to the safety assessment of the enzyme acyltransferase produced by a genetically modified strain of *B. licheniformis* to be used as a processing aid.

Two submissions were received on the Assessment Report. These are summarised in **Attachment 2**.

Submitters' comments have been taken into account in FSANZ's decision, with specific issues discussed below.

10.2 Issues raised in submissions

10.2.1 Enzyme nomenclature

The New Zealand Ministry of Agriculture and Forestry (MAF) suggested the name of the enzyme in the Code should be 'Glycerophospholipid cholesterol acyltransferase, protein engineered variant EC 2.3.1.43', stating this name better describes the enzyme and aligns with that used in the USFDA GRAS notice and CCFA Inventory of Processing Aids (IPA).

10.2.1.1 Response

FSANZ acknowledges and agrees that the suggested name better describes the enzyme than that originally proposed and is consistent with both the GRAS and IPA listings. The draft variation provided at **Attachment 1A** reflects the revised enzyme name.

10.2.2 Enzyme methodology

Queensland Health notes minimal information was provided regarding the methodology for determining enzyme activity, stating the kit-based method described would apply to total acyltransferase activity and not specifically to this genetically modified source. They requested further advice be provided to jurisdictions who have responsibility for monitoring and enforcing any amendments to the Code.

10.2.2.1 Response

FSANZ acknowledges that the requirement for analytical methods has been a longstanding concern for jurisdictions that ensure compliance with the Code.

Methods for determining enzyme activity are based on the reaction catalysed. The methodology provided by the Applicant (Appendix A3 in the Application) is based on the enzyme's ability to hydrolyse lecithin and liberate free fatty acids. As discussed in section 2.2.1 of SD1, the free fatty acids are then measured via a commercially available kit (NEFA C kit by WAKO GmbH). The rate of fatty acid liberation is then proportional to the activity of the enzyme.

Assays to determine enzyme activity are appropriate for monitoring conformance to product specifications during production of the enzyme preparation, but are normally unsuitable as a tool that jurisdictions can use to check compliance to the Code. Enzyme processing aids are not normally present in the final food and if they are present, they would only be in minute amounts and likely to be as inactive protein. Therefore, methodology which is based on measuring reaction rates catalysed by the enzyme is ineffective for final food analyses. Furthermore, enzyme processing aids are permitted according to good manufacturing practice (GMP); therefore no maximum limit exists in the Code to measure compliance against.

FSANZ therefore does not consider it necessary to provide further information regarding enzyme methodology.

10.2.3 Enzyme approval

Queensland Health has requested advice be provided on the expected timeline of the enzyme's approval process in Brazil.

10.2.3.1 Response

In order to approve an enzyme processing aid, FSANZ first conducts an independent, scientifically robust assessment of the safety and technological suitability of the enzyme for the proposed use. While permission for the enzyme in other countries is also noted in our assessment, we do not need to give consideration to the outcome of pending approvals.

FSANZ requested the Applicant provide an update on the enzyme's international regulatory status including that of applications in process. Advice received indicated the applications in Denmark and Brazil are yet to be finalised although no estimates were provided on expected timelines. Information was also unavailable from Brazilian authorities.

10.2.4 Cost Benefit assessment

Queensland Health have requested further advice be provided on how the cost benefit was determined, specifically with regard to the conclusion that there would be little or no predicted cost impost on jurisdictions, given an amendment to the Code is necessary.

10.2.4.1 Response

In developing food regulatory measures, FSANZ is required to consider impacts of the options on consumers, relevant food industries and governments. The regulatory impact assessment identifies and evaluates the costs and benefits arising from the regulation with the level of analysis commensurate to the nature of the proposal and significance of the impacts. The impacts of amending the Code to permit a processing aid are considered minor or machinery in nature as they are part of implementing a regulatory framework where their use is voluntary. In light of this, the OBPR have advised FSANZ that regulatory impact statements are not required for this type of application.

The proposed permission in the Code clearly identifies that this is a novel protein. Mandatory labelling is required should novel protein be present in the final food. Labelling costs are a consideration for manufacturers in determining the cost benefit of their voluntary use of a new enzyme. As noted in section 10.2.1.1, the analysis of foods for the presence or otherwise of the enzyme is ineffective, unnecessary and unsuitable as a compliance tool for jurisdictions.

In the cost benefit section of the Report, FSANZ stated that there is not predicted to be any significant cost impost for jurisdictions to determine compliance compared to current monitoring and compliance activities. Given that use of processing aids by manufacturers is voluntary and not subject to limits in the Code, it is unlikely that there would be new or additional compliance costs which exceed current jurisdictional responsibilities. It is noted that the OBPR has determined that no RIS is required for these types of applications.

10.3 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 allow acyltransferase as a permitted processing aid (enzyme) is unlikely to have a significant effect on international trade as the enzyme preparation complies with international standards for food enzymes as gazetted by JECFA and the FCC.

Notification to WTO under FSANZ's obligations under the WTO Technical Barriers to Trade or Sanitary and Phytosanitary Measures Agreements was not considered necessary.

Conclusion

11. Conclusion and Decision

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

The Report concludes that use of a protein engineered acyltransferase produced by genetically modified *B. licheniformis* as a processing aid is technologically justified and does not pose a public health and safety risk.

Existing labelling provisions are appropriate and enable consumers to have adequate information to make informed purchase choices.

FSANZ has concluded there are no misleading or deceptive conduct aspects to this assessment.

The Ministerial Council Policy Guidelines have been addressed in this assessment. The technological function of using the substance has been articulated and assessed as being met. Its use as proposed has been assessed as being safe and suitable.

An amendment to the Code giving permission for the use of this acyltransferase as a processing aid in Australia and New Zealand is recommended on the basis of the available scientific information.

The proposed draft variation is provided in **Attachment 1A**.

Decision

To approve a variation to Standard 1.3.3, subject to amendment, to permit the use of a protein-engineered variant of acyltransferase produced by a genetically modified *Bacillus licheniformis* as a processing aid.

11.1 Reasons for Preferred Approach

- An amendment to the Code approving the use of this acyltransferase as a processing aid in Australia and New Zealand is proposed on the basis of the available evidence 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.

- The source organism, *B. licheniformis* is regarded as non-pathogenic and non-toxigenic and has a safe history of use in production of food enzymes.

- Use of acyltransferase produced from a GM *B. licheniformis* as a processing aid is technologically justified and would be expected to provide benefits to food manufacturers in terms of product quality, yield and manufacturing processes. Potential benefits may also exist for consumers in the provision of products with improved and consistent quality.

- Permitting use of the enzyme would not impose significant costs for government agencies, consumers or manufacturers.

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

- There are no relevant New Zealand standards.

12. Implementation and Review

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

ATTACHMENTS

1A. Draft variations to the *Australia New Zealand Food Standards Code* (at Approval)
1B. Draft variations to the *Australia New Zealand Food Standards Code* (at Assessment)
2. Summary of Public Submissions on the Assessment Report

Attachment 1A

Draft variation to the *Australia New Zealand Food Standards Code* (At Approval)

Food Standards (Application A1050 – Glycerophospholipid Cholesterol Acyltransferase as a Processing Aid) Variation

The Board of Food Standards Australia New Zealand gives notice of the making of this variation under section 92 of the *Food Standards Australia New Zealand Act 1991*. The Standard commences on the date specified in clause 3 of this variation.

Dated DATE OF GAZETTAL

Standards Management Officer
Delegate of the Board of Food Standards Australia New Zealand

1 Name

This instrument is the *Food Standards (Application A1050 – Glycerophospholipid Cholesterol Acyltransferase as a Processing Aid) Variation*.

2 Variation to Standards in the *Australia New Zealand Food Standards Code*

The Schedule varies the Standards in the *Australia New Zealand Food Standards Code*.

3 Commencement

These variations commence on **DATE OF GAZETTAL**.

SCHEDULE

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

Glycerophospholipid cholesterol acyltransferase, protein engineered variant EC 2.3.1.43	*Bacillus licheniformis,* containing the gene for glycerophospholipid cholesterol acyltransferase isolated from *Aeromonas salmonicida* subsp. *salmonicida*

Attachment 1B

Draft variation to the *Australia New Zealand Food Standards Code* (At Assessment)

Section 94 of the FSANZ Act provides that standards or variations to standards are legislative instruments, but are not subject to disallowance or sunsetting

[1] ***Standard 1.3.3*** *is varied by inserting in the* Table to clause 17 –

Acyltransferase, protein engineered variant EC 2.3.1.43	*Bacillus licheniformis,* containing the gene for acyltransferase isolated from *Aeromonas salmonicida* subsp. *salmonicida*

Attachment 2

Summary of Public Submissions on the Assessment Report

Two submissions were received during the public consultation period for the Assessment Report, both from government jurisdictions.

A summary of the submissions is provided in the Table below.

Submitter	Group	Comments
New Zealand Ministry of Agriculture and Forestry (MAF)	Government	• Support the Application • Suggests the drafting entry be amended to Glycerophospholipid cholesterol acyltransferase, protein engineered variant EC 2.3.1.43, to more accurately reflect the enzyme nomenclature
Queensland Health	Government	• The submission is a 'whole of Queensland government response' and notes that although other Queensland government agencies indicated no issues relative to the Application, Queensland Health request further information on a number of concerns. – further advice on methodology for determining the activity of this enzyme – advice on the timeline relative to the enzyme's approval process in Brazil – further advice on the cost/benefit analysis undertaken