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

10-05
7 December 2005

DRAFT ASSESSMENT REPORT

APPLICATION A519

LIPASE FROM *PENICILLIUM ROQUEFORTII* AS A PROCESSING AID (ENZYME)

DEADLINE FOR PUBLIC SUBMISSIONS: 6pm (Canberra time) 1 February 2006
SUBMISSIONS RECEIVED AFTER THIS DEADLINE
WILL NOT BE CONSIDERED
(See 'Invitation for Public Submissions' for details)

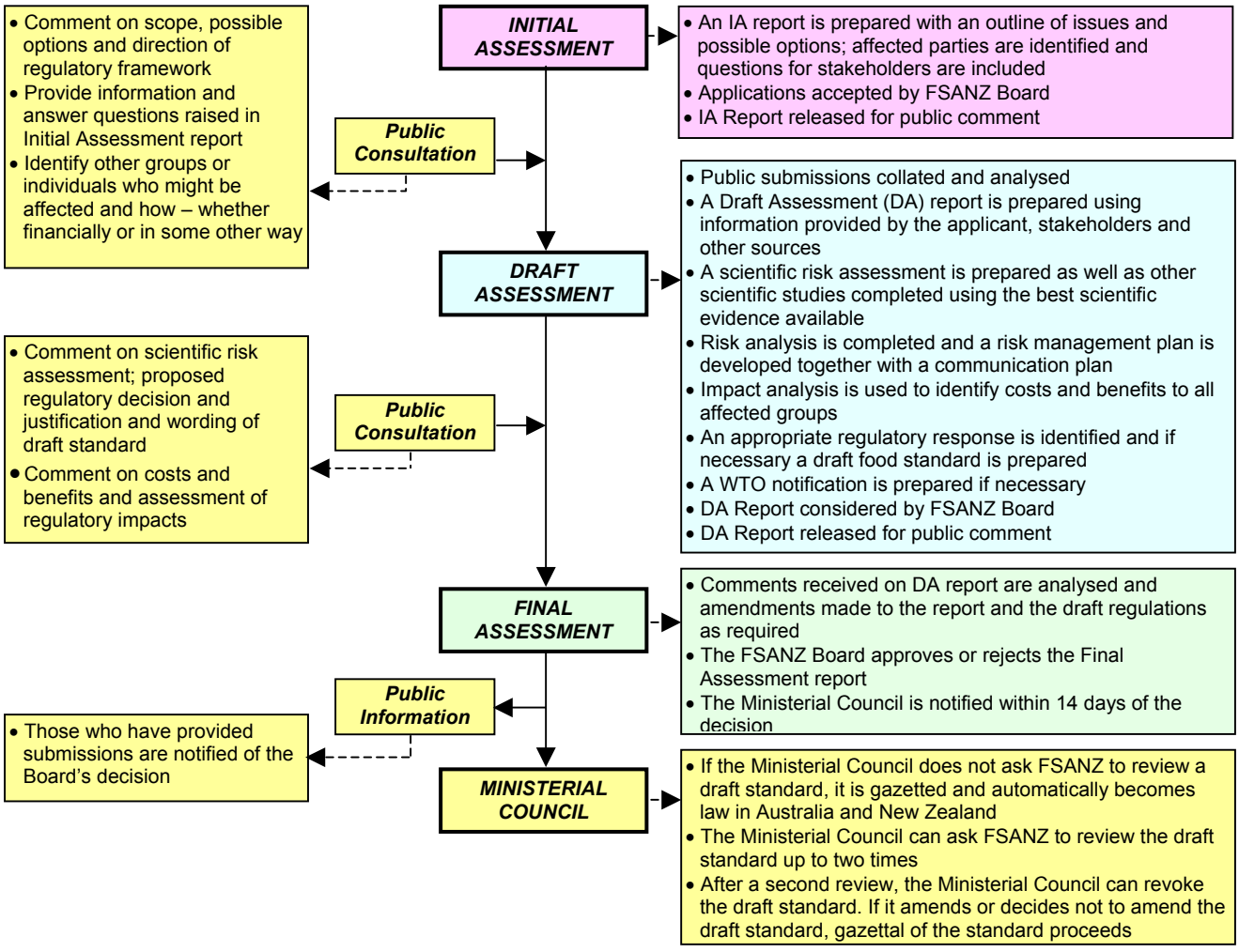
FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

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 a Draft Assessment Report of Application A519; and prepared a draft variation to the *Australia New Zealand Food Standards Code* (the Code).

FSANZ invites public comment on this Draft Assessment Report based on regulation impact principles and the draft variation to the Code 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 Final 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
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Submissions need to be received by FSANZ by 6pm (Canberra time) 1 February 2006.

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 and Statement of Reasons

FSANZ received an application on 6 November 2003, from Salkat Australia on behalf of Biocatalysts Ltd, to amend Standard 1.3.3 – Processing Aids of the *Australia New Zealand Food Standards Code* (the Code) to approve an enzyme, lipase, triacylglycerol (EC number [3.1.1.3]), as a processing aid. The new microbial fungal source for this enzyme is *Penicillium roquefortii.* The enzyme is not sourced from a genetically modified organism. An alternative name of the micro-organism source is *Penicillium roqueforti.*

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 lipase sourced from *P. roquefortii*, in the Code. The objective of this assessment is to determine whether it is appropriate to amend the Code to permit the use of lipase sourced from *P. roquefortii.*

The new enzyme has broad activity for hydrolysing triglycerides to short and medium chain fatty acids from the 1 and 3 positions on the glycerol molecule. The Applicant claims that the enzyme produces blue-cheese notes (odours) during dairy flavour production, which are desirable for certain types of cheese and cheese flavoured products.

The safety assessment of lipase from *P. roquefortii* concluded that:

- The source organism is non-pathogenic.
- The enzyme preparation complies with international specifications.
- In a sub-chronic study in rats, the no observed effect level (NOEL) was 2000 mg/kg bw per day, the highest dose tested.
- The enzyme preparation produced no evidence of genotoxic potential in *in vitro* assays.

The enzyme preparation meets the international specifications for enzymes, in the Food Chemicals Codex (4th Edition, recently updated to the 5th Edition (2004)) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) Compendium of Food Additive Specifications (2001). The Applicant states the enzyme has been confirmed independently as self-affirmed GRAS (Generally Recognized As Safe) in the USA. It is approved for use in Japan under the general permission given for 'lipase'.

The only regulatory options considered were to approve or not approve the use of the enzyme, lipase sourced from *P. roquefortii* as a processing aid. Approval of the Application provides advantages to manufacturers of modified cheeses and producers looking for specific cheese flavour profile which they can add to many different processed foods. There should be no added costs to government regulators or consumers.

Public comment on the Initial Assessment Report was sought from 25 May till 6 July 2005. Six submissions were received of which two supported the Application and three reserved comment until the Draft Assessment and one raised an issue which has been addressed.

From the available information, it is concluded that the use of lipase, triacylglycerol from *P. roquefortii* as a processing aid would not raise any public health and safety concerns, and is technologically justified.

Submissions are now invited on this report to assist FSANZ to complete the Final Assessment.

FSANZ Decision

Approval is proposed for the enzyme, lipase, triacylglycerol (EC [3.1.1.3]) from a new microbiological source, the fungus *Penicillium roquefortii*. Permission is proposed to be provided by adding this enzyme into the Table to clause 17 of Standard 1.3.3 – Processing Aids of the Code.

Statement of Reasons

The draft variation to Standard 1.3.3 – Processing Aids, thereby giving approval for the use of lipase, triacylglycerol sourced from *P. roquefortii* as a processing aid is proposed for the following reasons.

- Use of the enzyme does not raise any public health and safety concerns.
- Use of the enzyme is technologically justified since it has a role in the preparation of enzyme modified cheeses, with a specific flavour profile and for cheese flavours.
- The source organism, *P. roquefortii* is a well understood organism that is considered non-pathogenic.
- The proposed draft variation to the Code is consistent with the section 10 objectives of the FSANZ Act. In particular, it does not raise any public health and safety concerns, the safety assessment of the enzyme is based on the best available scientific evidence and it helps promote an efficient and internationally competitive food industry.
- The regulation impact assessment has concluded that the benefits of permitting use of the enzyme outweigh any costs associated with its use.
- The most cost-effective means to achieve what the Application seeks, namely permission to use lipase sourced from *P. roquefortii* as a processing aid, is a variation to Standard 1.3.3.

1. Introduction

FSANZ received an application on 6 November 2003, from Salkat Australia on behalf of Biocatalysts Ltd, to amend Standard 1.3.3 – Processing Aids of the Code to approve an enzyme, lipase, triacylglycerol (EC number [3.1.1.3]), as a processing aid. It is a Group 2 Application. Work started on this Application in the second Quarter of 2005.

This new microbial fungal source is *Penicillium roquefortii.* The enzyme is not sourced from a genetically modified organism. An alternative name of the micro-organism source is *P. roqueforti.* Both names are valid, but the name used in the Application (*P. roquefortii*) will be used in this report to refer to the source organism. The Safety Assessment Report uses *P. roqueforti* since this name is used in the title of many of the studies assessed.

The Applicant claims that this new enzyme has broad activity for hydrolysing triglycerides to short and medium chain fatty acids from the 1 and 3 glycerol positions. It is claimed to produce blue-cheese notes (odours) during dairy flavour production.

2. Regulatory Problem

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

The Table to clause 17 of Standard 1.3.3 contains a list of permitted enzymes of microbial origin. There are a number of approved sources of the enzyme, lipase, triacylglycerol, but not the source *P. roquefortii*. *P. roquefortii* is also not the source of any other approved enzymes in this Table.

FSANZ also has two similar applications from the same Applicant, Biocatalysts Ltd, which are now being assessed. These applications are seeking approval for other sources for the enzyme, lipase, triacylglycerol; A516 sourced from *Candida rugosa* and A517 sourced from *Mucor javanicus*.

3. Objective

The objective of this assessment is to determine whether it is appropriate to amend the Code to permit the use of lipase, triacylglycerol sourced from *P. roquefortii.*

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

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

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

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

4. Background

4.1 Historical Background

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

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

The Applicant claims that the main uses for this new enzyme will be in the dairy industry, specifically in the enzyme modified cheese area. Uses of lipases in the dairy industry include the flavour enhancement of cheeses, the acceleration of cheese ripening, the manufacturing of cheese-like products and cheese flavours, plus the lipolysis (cleavage of the triglycerides) of butterfat and cream[2].

The traditional sources of lipases used for cheese manufacture and for cheese flavour enhancement are from animal tissues, such as pancreatic glands (bovine and porcine) and the pre-gastric tissues of young ruminants (kid, lamb and calf)[2]. These sources of lipases are listed in the Table to clause 15 of Standard 1.3.3 of the Code (lipase EC [3.1.1.3], sourced from bovine stomach; salivary glands or forestomach of calf, kid or lamb; porcine or bovine pancreas).

There has also been a large range of microbial lipase preparations, which are non-animal derived enzymes, developed for the cheese industry. Such enzymes have advantages by being Kosher approved as well as available for vegetarian consumers.

[1] Pandey, A.; Benjamin, S.; Soccol, C.R.; Nigam, P.; Krieger, N. and Soccol, V.T. (1999) The realm of microbial lipases in biotechnology, *Biotechnol. Appl. Biochem.* **29**,:119-131.
[2] Anna University – Chennai – India, Applications of Lipases
http://www.au-kbc.org/beta/bioproj2/uses.html

4.2 Micro-organism nomenclature

During the assessment it was found that there are two names used to refer to the micro-organism. Both *P. roquefortii* and *P. roqueforti* are used in the literature. An assessment was made to check if the two names referred to the same micro-organism, and if so which name should be used. The conclusion of the nomenclature assessment in the microbiological literature was that both names are acceptable and the use of either name will not lead to confusion in the scientific community.

The organism is referred in Europe as *P. roqueforti,* as evidenced by the largest culture collection in Europe (the Centraalbureau voor Schimmelcultures, or the CBS culture collection). The same organism is referred in North America as *P. roquefortii*, as evidenced by the largest culture collection in North America (the American Type Culture Collection, or the ATCC collection).

Since either name can be used, and both names refer to the same organism, the name used in the Application, that is *P. roquefortii*, is used for most of this Report. As well an editorial note in the legal drafting to Standard 1.3.3 is proposed to be written to indicate that both spellings are acceptable and refer to the same organism (see **Attachment 1**).

5. Relevant Issues

5.1 Risk assessment

The enzyme is used as a processing aid only, and is not expected to be present in the final food as a result of most food uses. Any residue in the final food would be in the form of inactivated enzyme, which would be metabolised like any other protein.

Five studies relevant for the safety assessment were submitted in support of this Application. These were:

a) a pathogenicity study of *P. roquefortii* in mice;
b) an acute toxicity study in mice and rats;
c) a 90-day sub-chronic oral toxicity study in rats;
d) a reverse mutation test in bacteria; and
e) a chromosomal aberration test in cultured Chinese hamster cells.

The safety assessment of lipase from *P. roquefortii* concluded that:

- The source organism is non-pathogenic.
- The enzyme preparation complies with international specifications.
- In a sub-chronic study in rats, the No Observed Effect Level (NOEL) was 2000 mg/kg bw per day, the highest dose tested.
- The enzyme preparation produced no evidence of genotoxic potential in *in vitro* assays.

From the available information, it is concluded that the use of lipase from *P. roquefortii* as a processing aid in food would not raise any public health and safety concerns. The Safety Assessment Report is at **Attachment 3**.

5.2 Nature of the enzyme

The enzyme is called lipase, triacylglycerol in the Table to clause 17 of Standard 1.3.3 of the Code. Its common name is lipase, with other alternative names being triacylglycerol acylhydrolase and phospholipase.

It has the Enzyme Commission (EC) number of [3.1.1.3] and a CAS number of 9001-62-1. This is a different enzyme to another lipase listed in the Table to clause 17, which is called lipase, monoacylglycerol EC [3.1.1.23].

The enzyme is produced by fermentation of the microbial fungal source *P. roquefortii*. The enzyme preparation is a white powder. The Applicant claims the enzyme preparations meet the international enzyme specifications in the Food Chemicals Codex, 4th Edition, 1996[3] and the FAO/WHO Joint Expert Committee on Food Additives (JECFA), in the Compendium of Food Additives Specifications, Vol 1 Annex 1, FAO 1992 (Addendum 9, 2001)[4].

There are no dietary or nutritional implications for approval of this enzyme. Any residues in the final food would be inactivated enzyme which would be metabolised like any other protein. It is important for the manufacturer of EMC that the enzyme is inactivated by heat or else the desired flavour profile will continue to change, which would be unacceptable.

5.3 Efficacy and technological justification

Lipases are enzymes that catalyse the cleavage of triglycerides to fatty acids. The Applicant claims lipase sourced from *P. roquefortii* has broad activity for hydrolysing triglycerides to short and medium chain fatty acids from the 1 and 3 glycerol positions. Its specific proposed use is to produce blue-cheese flavours.

The Applicant claims that the main uses for this new enzyme will be in the dairy industry, specifically in the EMC area. Uses of lipases in the dairy industry include the flavour enhancement of cheeses, the acceleration of cheese ripening, the manufacturing of cheese-like products and cheese flavours, plus the lipolysis (cleavage of the triglycerides) of butterfat and cream[2].

EMC is produced from a reasonably recent technology that has been developed in the food industry. Cheese precursors are incubated with enzymes at elevated temperatures to produce a more concentrated cheese type flavour which can then be used in other products (such as cheese, dips, sauces, dressings, soups, snacks etc). Lipases from different source organisms have different properties and can produce different flavour profiles. Use of this technology allows cheeses to be produced more quickly and economically than traditional cheese making processes. That is, it allows manufacturers to add controlled amounts of specific cheese flavours to replicate natural cheese ripened flavours.

The Application states that the enzyme is being evaluated for use in dairy products by New Zealand dairy companies.

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[3] Food Chemicals Codex, (1996). National Academy of Sciences, Food and Nutrition Board, Committee on Food Chemicals Codex, 4th edition, National Academy Press, Washington DC (recently updated to the 5th Edition (2004)).

[4] Joint FAO/WHO Expert Committee on Food Additives (JECFA) Compendium of Food Additive Specifications (2001). General specifications and considerations for enzyme preparations used in food processing. FAO Food and Nutrition Paper 52, Addendum 9, pp37-39.

The Food Technology Report **(Attachment 4)** provides more information about the purpose and use of the enzyme and concludes that the enzyme is technologically justified to produce unique cheese flavours for the food industry and specifically for enzyme modified cheese manufacture.

5.4 Other international regulatory standards

The Applicant states that the enzyme has been confirmed independently as self-affirmed Generally Recognized As Safe (GRAS) from this source in the USA. Under current US FDA (Food and Drug Administration) regulations there is no requirement for the FDA to confirm the GRAS status. It is up to the enzyme manufacturers to ensure the safety of their products. The enzyme is approved for food use in Japan under the general approval given for 'lipase'.

5.5 Issues addressed from submissions

5.5.1 Proposed uses of the enzyme

One submission to the Draft Assessment Report expressed the view that 'it is not known whether the conclusion of the safety assessment would apply to all possible uses of the processing aid permitted by the proposed change in the Standard'.

5.5.1.1 Response

The enzyme, lipase, triacylglycerol, catalyses the cleavage of triglycerides to fatty acids and glycerol. As such, it has limited uses in food preparation and the only identified use is in cheese manufacturing. The studies that have been conducted on the enzyme are considered adequate to address potential safety concerns, which in the case of an enzyme, are focused largely on possible contaminants rather than on the safety of the protein itself, which would be expected to be readily digested in the GI tract. These safety studies did not indicate any cause for concern in relation to this enzyme, even when the enzyme was administered to animals at significantly higher exposure levels than those to which humans would be exposed. Given these results, there would be no reason to be concerned even if the enzyme was found to have some other uses in food production. Similar lipase enzymes are not restricted in their usage.

5.5.2 International permissions

A submitter raised two questions relating to international permissions for use of the enzyme for which they sought a response in the Draft Assessment Report. They questioned the self-affirmed US GRAS status, as well as whether the Japanese approval for the specific enzyme is given by the general permission for 'lipase'. These issues were raised with the Applicant seeking their confirmation. The response is as explained in section 5.4.

5.5.3 Mycotoxins and safety of workers in blue cheese manufacture

A submitter raised two issues related to the safety of the source, *P. roquefortii* for the enzyme. The submitter understood that *P. roquefortii* is safe for use in cheese manufacture. However they understood the fungus produces mycotoxins and asked that this be addressed in the assessment. The submitter further asked about the safety of workers in blue cheese factories with possible respiratory problems.

The Safety Assessment Report (**Attachment 3**) investigated the issue of the production of mycotoxins by the production organism, *P. roquefortii.*

PR toxin and roquefortine appear to be the most toxic of the mycotoxins produced by *P. roquefortii*. PR toxin, one of the most potent mycotoxins, is unstable and deteriorates rapidly, so apparently under normal production conditions does not pose a health effect problem. Roquefortine had been recovered from blue cheese at low levels and there have been no reported adverse effects from consumption of the cheese There is no evidence that roquefortines are formed in significant levels in cheese. They occur in infected feed grain, wilted grasses or whole-crop maize silages . Therefore, the formation of mycotoxins produced by *P. roquefortii* when used for the production of lipases is considered to be a low public health and safety risk.

The second question relating to the respiratory safety of workers in blue cheese manufacture is considered an occupational health and safety issue, which is outside the scope of FSANZ and this Application.

5.3 Risk management

The risk assessment performed for the enzyme lipase, triacylglycerol sourced from *P. roquefortii* as a processing aid in food concluded that its use would not raise any public health and safety concerns.

There are no dietary modelling issues with the use of lipase triacylglycerol sourced from *P. roquefortii* since the enzyme is not usually expected to be present in the final food and any residue will be inactivated during subsequent processing and would be metabolised as any other protein.

The risk management decision for enzymes, which act as processing aids and have been assessed and found to perform a technological function and not raise any public health and safety concerns is to regulate them as permitted enzymes in Standard 1.3.3 – Processing Aids of the Code. Since the source for this enzyme is of microbial origin, approval will be listed in clause 17 – Permitted enzymes of microbial origin. The enzyme name, EC number and source are proposed to be listed. This proposed drafting is listed in **Attachment 1**.

A separate sentence will also be added to the editorial note for clause 17 to say that an alternative spelling of the source organism is *P. roqueforti.*

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.

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

Option 1. **Not approve** the use of lipase, triacylglycerol sourced from *P. roquefortii* as a processing aid.

Option 2. **Approve** the use of lipase, triacylglycerol sourced from *P. roquefortii* 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 dairy companies who produce enzyme modified cheese and cheese flavours;

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.

7.2.1 Option 1

There are no perceived benefits to industry, government regulators or consumers if this option is taken.

There are disadvantages to those food industries, specifically dairy manufacturers and food manufacturers who wish to use cheese flavours in their products, if this option is taken.

7.2.2 Option 2

There are advantages to dairy industry manufacturers of cheese and EMC, as well as food industries who wish to use different cheese flavours in their food products.

There should also be added variety of food products and flavours for consumers. As well consumers with vegetarian and Kosher certification requirements for cheese and cheese flavoured products should have an increased range of products.

There should be no added costs to government food regulators or consumers.

Option 2, which supports the approval of lipase, triacylglycerol sourced from *P. roquefortii* as a processing aid is the preferred option, since it has advantages for the food industry and consumers but has no significant cost for government regulators, consumers or food manufacturers.

8. Consultation

8.1 Public consultation

Public comment on the Initial Assessment Report for this Application was sought from 25 May till 6 July 2005. Six submissions were received of which two supported the Application and three reserved comment until the Draft Assessment and one did not state a position but raised an issue which has been addressed in an earlier section (section 5.5). Other issues raised in submissions have also been addressed in section 5.5. **Attachment 2** summarises the submissions received during this first round of public comment.

FSANZ is seeking further public comment on this Draft Assessment Report to assist in assessing this application at Final Assessment.

Comments that addressed the following topics would be useful:

- safety considerations of the enzyme and source;
- technological justification, including any supporting letters from possible dairy industries that have an interest in using the enzyme;
- any other scientific aspects, including use and approval of the enzyme in other nations; and
- various costs and benefits of its use, including how various food industries anticipate they may use the enzyme and in which foods.

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 lipase, triacylglycerol sourced from *Penicillium roquefortii* is unlikely to have a significant effect on international trade as most countries do not regulate enzymes as processing aids in a separate standard as Australia and New Zealand. Also when it is used as a processing aid there is unlikely to be any enzyme remaining in the final food and no requirement to label any final food. The enzyme preparations are consistent with the international specifications for food enzymes of the Food Chemicals Codex (5th Edition, 2004) and the JECFA Compendium of Food Additives Specifications so there does not appear to be a need to notify the WTO under either the Technical Barrier to Trade (TBT) or Sanitary and Phytosanitary Measures (SPS) Agreements.

9. The Decision

Approval is proposed for the enzyme, lipase, triacylglycerol (EC [3.1.1.3]) from a new microbiological source, the fungus *P. roquefortii*. Permission is proposed to be provided by adding this enzyme into the Table to clause 17 of Standard 1.3.3 – Processing Aids of the Code.

The draft variation to Standard 1.3.3 – Processing Aids, thereby giving approval for the use of lipase, triacylglycerol sourced from *P. roquefortii* as a processing aid is proposed for the following reasons.

- Use of the enzyme does not raise any public health and safety concerns.

- Use of the enzyme is technologically justified since it has a role in the preparation of enzyme modified cheeses, with a specific flavour profile and for cheese flavours.

- The source organism, *P. roquefortii* is a well understood organism that is considered non-pathogenic.

- The proposed draft variation to the Code is consistent with the section 10 objectives of the FSANZ Act. In particular, it does not raise any public health and safety concerns, the safety assessment of the enzyme is based on the best available scientific evidence and it helps promote an efficient and internationally competitive food industry.

- The regulation impact assessment has concluded that the benefits of permitting use of the enzyme outweigh any costs associated with its use.

- To achieve what the Application seeks, namely permission to use lipase sourced from *Penicillium roquefortii* as a processing aid, there are no alternatives that are more cost-effective than a variation to Standard 1.3.3.

ATTACHMENTS

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

Attachment 1

DRAFT VARIATION TO THE *AUSTRALIA NEW ZEALAND FOOD STANDARDS CODE*

To commence: On gazettal

[1] ***Standard 1.3.3*** *of the Australia New Zealand Food Standards Code is varied by –*

[1.1] *inserting in the* Table to clause 17, *for the enzyme* Lipase, triacylglycerol EC [3.1.1.3], *the source –*

Penicillium roquefortii

[1.2] *inserting in the* Editorial note *following the* Table to clause 17 –

Penicillium roquefortii is also known as *Penicillium roqueforti.*

Attachment 2

Summary of submissions

Round one

Submitter Organisation	Name
Queensland Health	Gary Bielby
Victoria Department of Human Services	Victor Di Paola
New Zealand Food Safety Authority	Carole Inkster
New South Wales Food Authority	Kelly Boulton
Western Australian Food Advisory Committee	Paul Van Buynder
Australian Food and Grocery Council	Kim Leighton

Submitter	Position	Comments
Queensland Health	Reserve its position until the safety assessment	It did not accept nor reject the Application at this stage, but will review once it has assessed the safety assessment (the Draft Assessment Report). It did have some comments which it believes should be addressed in the safety assessment. It understands *Penicillium roquefortii* is safe for use in cheese manufacture. However it understands the fungus produces mycotoxins. Is there a safety issue with the mycotoxins, relevant to the enzyme? It also asked about the safety of workers in the blue cheese factories, with possible respiratory problems.
Victoria Department of Human Services	Supports	It supports option 2, to approve the use of the enzyme.
New Zealand Food Safety Authority	No position stated	It made two comments which it would like addressed in the Draft Assessment. It was unable to verify the self-affirmed US GRAS status. As well it would like confirmation if this particular new particular fungal source of the lipase enzyme is permitted in Japan, rather than the general approval for lipase.
New South Wales Food Authority	Did not state a position	The Application proposes general approval for use in all foods. However it has concerns about whether the conclusion that the enzyme is safe for use in food (since the enzyme would be inactivated in the final product) would apply to all possible uses of the enzyme.
Western Australian Food Advisory Committee	Reserve its position until the safety assessment	It will wait to assess the safety, toxicological and allergenic data as part of the safety assessment in the Draft Assessment Report. It did note that the enzyme has possible applications in several food sectors, in particular dairy, for lipases from non-animal and non-genetically modified organisms.
Australian Food and Grocery Council	Supports	It believes FSANZ will determine that the enzyme is safe, and technologically justified. It believes the enzyme should be approved subject to an appropriate safety assessment (as part of the Draft Assessment).

Attachment 3

Safety assessment report

Application A519 – Lipase sourced from *Penicillium roqueforti*

Summary and Conclusion

Application A519 seeks approval for the use of lipase triacylglycerol from a non-genetically modified *Penicillium roqueforti* as a processing aid.

The enzyme is used as a processing aid only, and is not expected to be present in the final food. Any residue would be in the form of inactivated enzyme, which would be metabolised like any other protein.

The safety assessment of lipase from *P. roqueforti* concluded that:

- The source organism is non-pathogenic.
- The enzyme preparation complies with international specifications.
- In a sub-chronic study in rats, the NOEL was 2000 mg/kg bw per day, the highest dose tested.
- The enzyme preparation produced no evidence of genotoxic potential in *in vitro* assays.

From the available information, it is concluded that the use of lipase from *P. roqueforti* as a processing aid in food would not raise any public health and safety concerns.

1 Introduction

Application A519 seeks approval for the use of lipase triacylglycerol from a non-genetically modified *P. roqueforti* as a processing aid. The name of the source organism used in this Safety Assessment Report is *P. roqueforti* (rather than *P. roquefortii*, which is used in the rest of the report) since that is the name used in most of the safety studies assessed.

The enzyme is used as a processing aid only, and is not expected to be present in the final food. Any residue would be in the form of inactivated enzyme, which would be metabolised like any other protein.

Five studies relevant for the safety assessment were submitted in support of this application. These were: a) a pathogenicity study of *P. roqueforti* in mice, b) an acute toxicity study in mice and rats c) a 90-day sub-chronic oral toxicity study in rats, d) a reverse mutation test in bacteria, and e) a chromosomal aberration test in cultured Chinese hamster cells.

2 The source (production) organism – *Penicillium roqueforti*

The safety of the production organism is an important consideration in the safety assessment for enzymes used as a processing aid.

In application A519 the approval is sought for the use of lipase from a non-genetically modified *P. roqueforti* as a processing aid.

One pathogenicity study on *P. roqueforti* was submitted that is summarised below.

P. roqueforti is a common fungus, which is widespread in nature and can be isolated from soil, decaying organic substances and plant parts. The major industrial uses of this fungus are for the production of blue cheeses, flavouring agents, antibacterials, polysaccharides, proteases and other enzymes (US Environmental Protection Agency 1997).

The pathogenic potential of *P. roqueforti* is very low, even as an opportunistic pathogen (US Environmental Protection Agency 1997). The major human health concern for *P. roqueforti* is its ability to produce mycotoxins. Many of the strains of *P. roqueforti* isolated from commercial blue cheeses as well as from mouldy grains and nuts have been shown in the laboratory to produce mycotoxins. These mycotoxins include isofumigaclavin C, penicillic acid, PR toxin, patulin, botryodiploidin and roquefortine. Some of these mycotoxins are produced by *P. roqueforti* strains used for cheese production and some have been detected in small amounts in the cheese itself. PR toxin and roquefortine appear to be the most toxic of the mycotoxins produced by *P. roqueforti*. Other mycotoxins produced by this organism appear to be less toxic and of low concern. An LD_{50} in rats has been reported for roquefortine as 1520 mg/kg ip. PR toxin has been shown to cause decreased motor activity and respiration rates, and hind leg weakness in mice and rats. It has also been shown to be lethal in rats and mice at relatively high intraperitoneal doses (US Environmental Protection Agency 1997).

Conditions conducive to the production of mycotoxins by *P. roqueforti* include a medium of high C/N ratios (usually with the medium supplemented with sucrose), growth of the fungus on the surface of the medium presumably due to the high oxygen content, and growth of the fungus in stationary phase. The production of toxins vary between strains of *P. roqueforti*: under specified conditions some strains produce mycotoxins while others do not (US Environmental Protection Agency 1997).

PR toxin, one of the most potent mycotoxins, is unstable and deteriorates rapidly, so apparently under normal production conditions does not pose a health effect problem. Roquefortine had been recovered from blue cheese at low levels and there have been no reported adverse effects from consumption of the cheese (US Environmental Protection Agency 1997). There is no evidence that roquefortines are formed in significant levels in cheese. They occur in infected feed grain, wilted grasses or whole-crop maize silages (European Mycotoxin Awareness Network 2005).

Pathogenicity study on *Penicillium roqueforti* in mice (anonymous, 1994)

Test material	spores of *Penicillium roqueforti*
Vehicle material	saline
Test Species	S1c:ICR female mice (5-10 animals/dose)
Dose	0, 2×10^4, 2×10^5, 2×10^6 cells/mice (intravenously administration)
GLP/guidelines	GLP statement was not included; in accordance with the sensitivity test of filamentous fungi specified in the Development of Method for safety evaluation of animal and micro-bacterial feeds, published by Agriculture, Forestry and Fisheries Research Council Secretariat, Japan.

Groups of 5-10 female mice received single doses of a spore suspension of *P. roqueforti* administered intravenously. The animals were observed for 14 days post-dose. At day 14 the animals were sacrificed and necropsy was performed. Brain, liver and kidneys were assessed for histopathology and viable spores. No clinical signs and mortality was observed. Viable fungi were found in the brain, liver and kidney. Dose dependency was observed in the liver and kidney. In the brain, viable fungi were only observed at the highest dose. Histopathology revealed a dose dependent increase in slight focal necrosis in the liver in 2/10 mice of the 2 x 10^5 cells/mice group and 4/10 of the 2 x 10^6 cell/mice group. Spores emerged in the sinusoid of the liver and the afferent arteriole of the kidney without evidence of budding.

The study indicated that *P. roqueforti* remained viable for 14 days in the mouse when a large dose was inoculated. The spores were only observed in the capillary system without evidence of budding or histopathological alteration in peripheral tissue. No mortality or severe changes in histopathological changes were observed. In conclusions, spores of *P. roqueforti* inoculated into the vein are viable in mice, but remain in the original form.

Furthermore, the exposure through the use of *P. roqueforti* as source for the production of lipase would be negligible (<100 CFU/g preparation). Therefore the source is considered non-pathogenic.

3 Purity of enzyme preparation and proposed specifications

Historically, enzymes used in food processing have been found to be non-toxic, and the main toxicological consideration is in relation to possible contaminants. The production organism in this case is non-toxic and non-pathogenic. The detailed specifications from the source to which the preparation was found to conform are shown in Table 1. This is consistent with the recommended purity specifications for food-grade enzymes (JECFA, 2001; Food Chemical Codex, 2004). *P. roqueforti* can produce the mycotoxins PR toxin and roquefortine. No specific tests for these mycotoxins were performed, however, JECFA or the Food Chemical Codex have no specifications for these substances.

Table 1: Complete specification of lipase sourced from *Penicillium roqueforti*

Criteria	Specification
Lipase activity (U/g)	3,000
Total viable count (cfu/g)	<50,000
Total coliforms (cfu/g)	<30
Salmonella (in 25 g)	Negative by test
Escherichia Coli (in 25 g)	Negative by test
Antibiotic activity	Negative by test
Heavy metals as Pb (mg/kg)	<30
Lead (mg/kg)	<5
Arsenic (mg/kg)	<3

4 Evaluation of the safety studies of lipase sourced from *Penicillium roqueforti*

4.1 Acute study

Oral acute toxicity tests in mice and rats (Murata, 1988)

Test material	Lipase R produced by *Penicillium roqueforti*, activity 16,760 units/g, Lot No. LRF-N40-001
Vehicle material	Distilled water
Test Species	SlC:ddY female and male mice and Slc:SD male and female rats (10 animals/sex/dose)
Dose	0, 500, 1000, 2000 mg/kg bw
GLP/guidelines	Quality assurance statement included; in accordance with the Guidelines of toxicity studies issued by the Ministry of Health and Welfare of Japan.

Groups of 10 male and 10 female mice and rats received single doses of lipase AY administered orally by gavage and were observed for mortality, morbidity, and clinical signs for 14 days post-dose. Body weights were measured prior to dosing, at day 1, 2, 3, 7, 10 and 14. At day 15 the animals were sacrificed and necropsy was performed. No clinical signs and mortality was observed. Body weights and necropsy revealed no treatment related effects.

4.2 Sub-chronic toxicity

90-day oral toxicity study in rats (anonymous, 1993)

Test material	Lipase R produced by *Penicillium roqueforti*, activity 16,760 units/g, lot no. LRF-N40-001
Control and vehicle material	Sterile distilled water
Test Species	Crj : CD (SD) rats 10 males and females per test dose; administration by gavage
Dose	0, 500, 1000, 2000 mg lipase/kg bw per day
GLP/guidelines	No GLP or quality assurance statements; Guideline for Toxicity Studies in Drugs prescribed by the Ministry of Health and Welfare, Japan

Study conduct

Groups of rats (10/sex/group) were treated with lipase by gavage at 0, 500, 1000 or 2000 mg/kg bw per day for 13 weeks. In two additional groups (10/sex/group) after 13 weeks of treatment at 0 and 2000 mg/kg bw per day, a four-week recovery period was added.

Clinical observations were recorded daily. Bodyweight and food consumption were recorded twice weekly; urinalysis in week 10-12 of treatment; ophthalmology, haematology and blood biochemistry was performed at the end of treatment. At the end of the study, all animals were sacrificed and necroscopy performed (gross examination, organ weights). Histopathology on selected organs was performed in the control and high dose group.

Results

Two females died from the 1000 mg/kg bw group on day 75. Necropsy revealed pulmonary haemorrhage in lungs, due to erroneous administration. No dose related mortality was observed. No dose related effects were observed on clinical signs, body weight, food consumption and ophthalmology. Urinalysis revealed dose related increases in urine specific gravity, reaching statistical significance in males at 1000 and 2000 mg/kg bw and in females at 500 and 2000 mg/kg bw. Sodium levels dose-related increased in both males (significant at 1000 and 2000 mg/kg bw) and females (significant at all dose levels). Potassium levels were elevated in both males (significant at 2000 mg/kg bw) and females (significant at all dose levels). Decreased pH values were observed after 90 days of treatment in both males and females. Urine chloride levels were increased statistically significantly in females at 500 and 2000 mg/kg bw/day. At the end of the recovery period no differences were seen between the control group and the 2000 mg/kg bw.

Plasma sodium and potassium levels were slightly increased in males and females at 2000 mg/kg bw/day compared to controls, however these values were within the normal range. No other treatment related effects were observed in haematology and biochemistry. Necropsy revealed no abnormal changes in all groups.

The authors of the study report explained the increase in sodium and potassium concentrations by the adding of inorganic salt during production of the test substance; the crude test substance contained 6.9% Na^+ and 0.7% K^+. This salt load could explain increased sodium and potassium concentration in urine; which is a normal physiological process, and in the recovery group no differences were observed.

The NOEL was 2000 mg/kg bw per day, based on the absence of adverse effects at the highest dose tested.

4.3 Genotoxicity studies

Reverse mutation test in bacteria (Mizutani, 1994)

Test article

The test article, raw Lipase R powder (Lot No LRFS04527, 991 u/g of lipase activity) was used. Lipase R is produced by *Penicillium roqueforti*.

Study design

Lipase was examined for mutagenic activity in four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537) and a strain of *Escherichia coli* (WP2urvA). Experiments were performed with or without metabolic activation using liver S9 fraction from chemically pre-treated rats. The study design is in accordance with Guidelines for in vitro mutagenicity testing, issued by the Ministry of Labor, Japan. A preliminary toxicity test was performed to select the concentrations of the test article to be used in the main assays. The study comprised of negative and positive controls with or without S9 metabolising system. Experiments for survival determination and estimation of mutant numbers were carried out in triplicates at each test point. Five doses of test substance were applied with 5 mg/plate as the highest dose level.

The sensitivity of the individual bacterial strains was confirmed by significant increases in the number of revertant colonies induced by diagnostic mutagens (sodium azide, 9-aminoacridine, 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide, 2-aminoanthracene, N-ethyl-N'-nitro-N-nitrosoguanidine).

Test	*Test material*	*Concentration*	*Test object*	*Result*
Reverse mutation (*In vitro*)	Lipase	First and second test: 0, 313, 625, 1250, 2500, 5000 μg/plate, with and without S9 mix	*S. typhimurium* TA98, TA100, TA1535, TA1537. *E. coli* WP2uvrA	-ve

Results and conclusion

No dose-related increases in mutation frequency were observed in the strains tested. It was concluded that lipase produced by *Penicillium roqueforti* did not exhibit mutagenic activity under the conditions of the test.

Chromosome aberration test in cultured Chinese hamster cells (Saigo, 1994)

Test article

The test article, Lipase R, lot no. RFS04527 was used. The activity was 991 U/g. Lipase R is produced by *Penicillium roqueforti*.

Study design

The potential of lipase R to damage the chromosomal structure was tested in an *in vitro* cytogenetics assay, using CHL/IU cells, derived from fibroblasts of the lung of Chinese hamsters. Tests were carried out in the presence and absence of S9 metabolic activation, over a broad range of doses. In the first experiment, in the absence of S9, the cells were treated for 22 or 46 hr. In an additional dose finding study in both the absence or presence of S9, the cells were treated for six hours and the harvest time was 16 or 40 hours after treatment stopped. The concentrations inducing 50% growth inhibition were estimated to be 800 μg/ml (22 hour treatment), 560 μg/ml (46-hour treatment), and over 5000 μg/ml (Metabolic activation test). Based on these results, the treatment levels in the main studies were 312.5, 625, 1250, and 2500 μg/ml in the absence of S9 using a continuous treatment until harvest at 22 or 46 hours; and 625, 1250, 2500 and 5000 μg/ml in the absence or presence of metabolic activation for six hours.

Test	*Test material*	*Concentration*	*Test object*	*Result*
chromosome aberration (*In vitro*)	Lipase R	312.5, 625, 1250, and 2500 μg/ml continuous treatment 0, 625, 1250, 2500, 5000 μg/plate, with and without S9 mix	CHL/IU cell line, derived from fibroblasts of lungs of Chinese hamster	-ve

Results and conclusion

Treatment did not produce biologically or statistically significant increases in the frequency of aberrant chromosomes at any concentration tested when compared to control values, either in the presence or absence of S9 metabolic activation. Positive controls, mitomycin-C (-S9) and benzo(a)pyrene (+S9), gave the expected increases in the frequency of aberrant metaphases, indicating the efficacy of the metabolic activation mix and the sensitivity of the test procedure.

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Attachment 4

Food technology report

A519 – LIPASE FROM *PENICILLIUM ROQUEFORTII* AS A PROCESSING AID (ENZYME)

Introduction

FSANZ received an Application from Biocatalysts Ltd to amend the *Australia New Zealand Food Standards Code* (the Code) to approve a new source, the fungus *Penicillium roquefortii*, for the enzyme lipase, triacylglycerol as a processing aid.

Lipase triacylglycerol

In the Table to clause 17 – Permitted enzymes of microbial origin of Standard 1.3.3 of the Code the name of this enzyme is lipase, triacylglycerol. Its common name is lipase, with other names including triacylglycerol lipase, triglyceride lipase and tributyrase. There already is approval for this enzyme in the Code but with a number of other sources, not *P. roquefortii*.

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

There is another lipase listed in Table to clause 17 of the Code, but this is called lipase, monoacylglycerol which is a different enzyme with an EC number of [3.1.1.23].

Lipase (EC [3.1.1.3]) is also listed in Table to clause 15 – Permitted enzymes of animal origin of the Code. This enzyme is sourced from bovine stomach; salivary glands or forestomach of calf, kid or lamb; porcine or bovine pancreas.

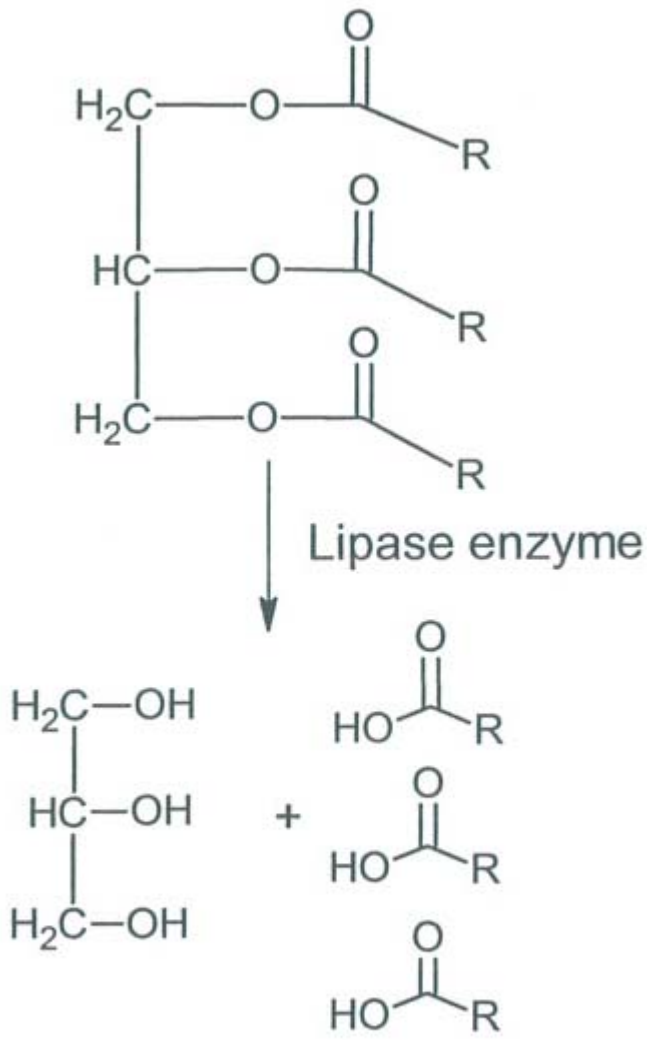
The enzyme for this Application is from a microbial source (the fungus *P. roquefortii*,) rather than an animal source.

The enzyme preparation is a white powder with pH stability between 5 and 8 and optimum pH of 7. The optimum temperature of use is 40ºC. It is thermally stable below 37ºC in an aqueous solution.

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

$$\text{Triacylglycerol} + H_2O \rightarrow \text{Diacylglycerol} + \text{a fatty acid anion}$$

In the Application it is stated that the enzyme attacks mainly the 1 and 3 triglyceride positions so it is able to cleave short and medium chain fatty acids from triglycerides(as indicated in the following schematic taken from the Application).



Technological justification

The Applicant states this enzyme acts on triglycerides in a significantly different way to other already approved lipase triacylglycerols and so enables the production of different cheese flavours.

A number of commonly used enzymes for cheese manufacture are produced from animal sources, as has been traditionally used. With this fungal source being a non-animal, microbial type it can be used to produce cheese for vegetarian consumers and consumers that prefer Kosher certification.

The Applicant claims lipase sourced from *P. roquefortii*, hydrolyses short and medium chain fatty acids from the number 1 and 3 positions of triglycerides. It is claimed to produce blue-cheese notes (odours) which are desirable for certain cheese types and cheese flavoured products.

The Applicant claims that the main uses for this new enzyme will be in the dairy industry, specifically in the enzyme modified cheese (EMC) area.

Uses of lipases in the dairy industry include the flavour enhancement of cheeses, the acceleration of cheese ripening, the manufacturing of cheese-like products and cheese flavours, plus the lipolysis (cleavage of the triglycerides) of butterfat and cream. Strong cheese flavours are also used in various convenience foods such as cheese dips, sauces, salad dressings, pizza topping and snack coatings (e.g. crisps and savoury biscuits).

EMC is a reasonably recent technology that has been developed in the food industry that incubates cheese precursors with enzymes at elevated temperatures to produce a more concentrated cheese type flavour which can then be used in other products. Bland flavoured immature cheese is incubated with enzymes to produce highly concentrated cheese flavours in very short time periods compared to the traditional slow cheese maturation. Lipases from different source organisms have different properties and so can produce different flavour profiles. Use of this technology allows cheeses to be produced quicker and more economically than traditional cheese making processes. That is, it allows manufacturers to add controlled amounts of specific cheese flavours to replicate natural cheese ripened flavours.

Production of the enzyme

The enzyme preparations are produced from standard enzyme manufacturing methods of fermentation of the micro-organism *P. roquefortii*. Fermentation feed stocks are sterilised prior to fermentation either by microfiltration (0.2 μm) or sterilisation (121°C for a minimum of 15 minutes). Final enzyme solutions are centrifuged to remove source organisms and concentrated by ultrafiltration.

Specification

The Application states that the enzyme preparations meet the international specifications for enzymes contained in the Food Chemical Codex, and the Joint FAO/WHO Expert Committee on Food Additives (JECFA), in the Compendium of Food Additives Specifications, Vol 1 Annex 1, FAO 1992 (Addendum 9, 2001). The specification below is taken from the Applicant's enzyme specification supplied.

Criteria	**Specification (meets or exceeds JECFA)**
Heavy Metals as Pb	not more than 30 ppm
Arsenic	not more than 3 ppm
Lead	not more than 5 ppm
Total viable count (cfu/g)	not more than 50,000
Total coliforms (cfu/g)	not more than 30
Mycotoxins	negative by test
Antibacterial activity	negative by test
Salmonella (/25 g)	negative by test
Escherichia coli (/25 g)	negative by test

Conclusions

The use of the enzyme lipase, triacylglycerol sourced from *Penicillium roquefortii* as a processing aid is technologically justified to produce unique cheese flavours for the food industry and specifically for enzyme modified cheese manufacture.

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http://us.expasy.org/cgi-bin/enzymes-search-ec

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