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

7-05
5 October 2005

DRAFT ASSESSMENT REPORT

APPLICATION A517

LIPASE FROM *MUCOR JAVANICUS* AS A PROCESSING AID (ENZYME)

DEADLINE FOR PUBLIC SUBMISSIONS: 6pm (Canberra time) 16 November 2005
SUBMISSIONS RECEIVED AFTER THIS DEADLINE
WILL NOT BE CONSIDERED
(See 'Invitation for Public Submissions' for details)

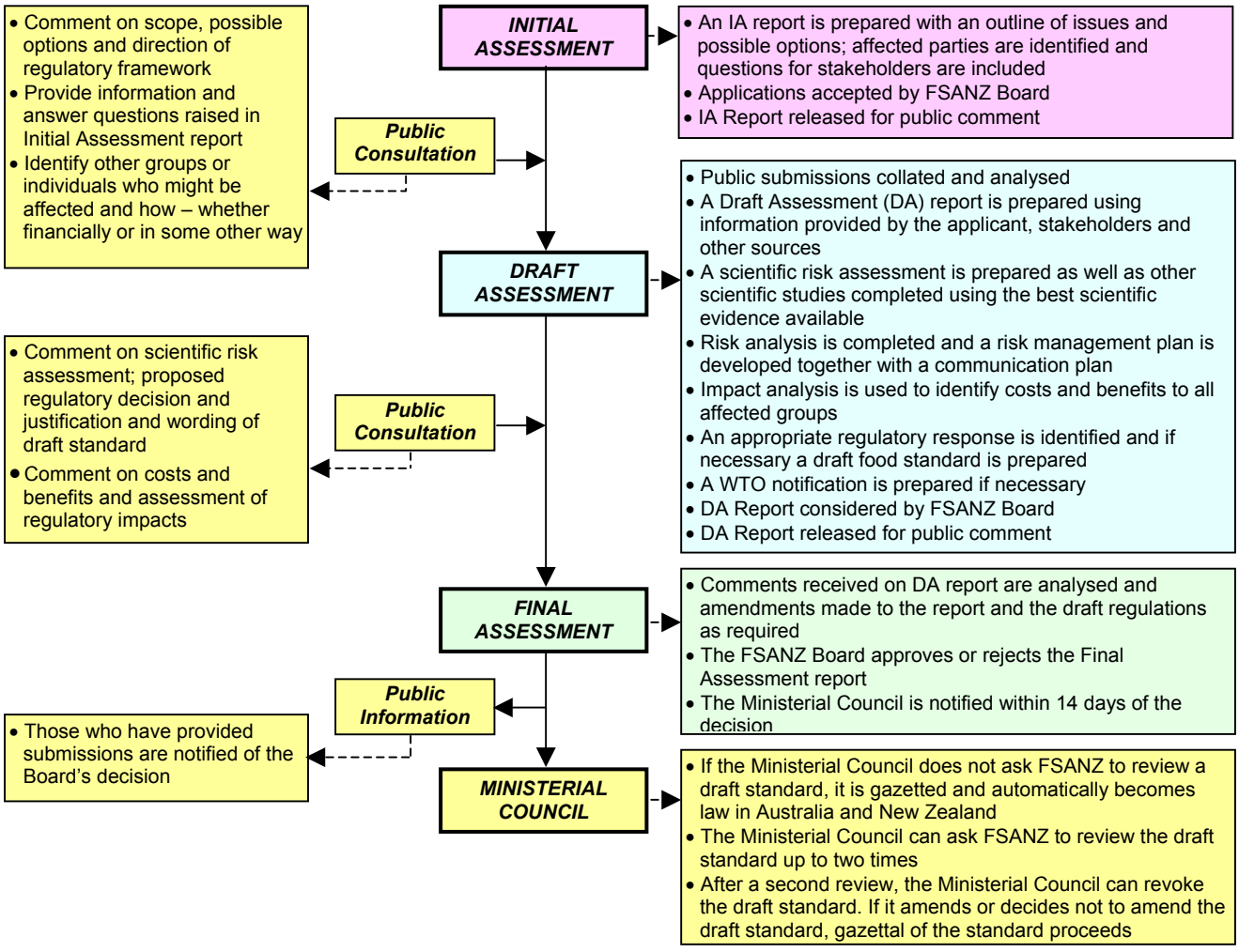
FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

FSANZ's role is to protect the health and safety of people in Australia and New Zealand through the maintenance of a safe food supply. FSANZ is a partnership between ten Governments: the Commonwealth; 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 Commonwealth, 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 Commonwealth, 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 A517; 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 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
www.foodstandards.govt.nz

Submissions need to be received by FSANZ by 6pm (Canberra time) 16 November 2005.

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

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

Assessment reports are available for viewing and downloading from the FSANZ website. Alternatively, requests for paper copies of reports or other general inquiries can be directed to FSANZ's Information Officer at either of the above addresses or by emailing info@foodstandards.gov.au.

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Executive Summary 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 Code to approve an enzyme, lipase, triacylglycerol (EC number [3.1.1.3]), as a processing aid. The enzyme is derived from a new microbial source, *Mucor javanicus,* which is a filamentous fungus. The enzyme is not sourced from a genetically modified organism. An alternative, more recent name for the microorganism is *M. circinelloides* f. *circinelloides.*

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 *M. javanicus*, 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 *M. javanicus.*

The main function of the proposed new enzyme is that it hydrolyses medium and long chain fatty acids, preferentially from the 1 and 3 positions of triglycerides. The fatty acids are used for the production of cheese flavours, which are desirable flavours for processed cheese.

The safety assessment of lipase sourced from *M. javanicus* concluded that:

- The source organism is non-pathogenic.
- The enzyme preparation complies with international specifications.
- The evidence from the available safety studies with the enzyme did not indicate any adverse effects.
- 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 *M. javanicus* as a processing aid in food would not raise any public health and safety concerns.

The enzyme preparation meets the international specifications for enzymes, namely the current Food Chemicals Codex (5th Edition, 2004) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) specifications (2001). The US Food and Drug Administration (FDA) has not questioned the self-affirmed GRAS (Generally Recognized As Safe) status of the enzyme. It is approved for use in Japan, under the general approval given for 'lipase'.

The only regulatory options considered were to approve or not approve the use of the enzyme, lipase sourced from *M. javanicus* 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 had been sought from 18 February to 31 March 2004. Three submissions were received, two supporting the Application and one waiting to see the Draft Assessment.

The Draft Assessment Report concludes that approval of lipase sourced from *Mucor javanicus* as a processing aid is technologically justified and does not raise any public health and safety concerns.

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, namely the fungus *Mucor javanicus*. Permission is given by adding this approval 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 *M. javanicus* as a processing aid is recommended for the following reasons.

- 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.

- 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, *M. javanicus* is a well understood organism (filamentous fungus) that is considered non-pathogenic.

- 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, triacylglycerol sourced from *M. javanicus* as a processing aid, there are no alternatives that are more cost-effective than 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. The enzyme is derived from a new microbial source, *M. javanicus,* which is a filamentous fungus. The enzyme is not sourced from a genetically modified organism. An alternative name for the microorganism is *M. circinelloides* f. *circinelloides*.

The Application was put onto the FSANZ Work Plan as a non-paid Application in May 2004 and work recommenced in the second quarter of 2005 (in line with the Work Plan).

The main function of the proposed new enzyme is hydrolysis of medium and long chain fatty acids, preferentially from the 1 and 3 positions of triglycerides. The fatty acids are used for the production of cheese flavours, which are desirable flavours for processed cheese. It can also be used to produce Enzyme Modified Cheese (EMC) with specific flavour profiles.

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 *M. javanicus*. *M. javanicus* (or *M. circinelloides* f. *circinelloides*) is also not the source of any other approved enzymes in this Table.

FSANZ also has a similar Application from the same Applicant, Biocatalysts Ltd, which is also at Draft Assessment. This Application is A516, which is seeking approval for another source for the enzyme, lipase triacylglycerol, sourced from *Candida rugosa*.

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 *M. javanicus* 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 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.

FSANZ will address the protection of public health and safety by ensuring that there are no significant health risks associated with approval of the new source of the lipase enzyme. This report has used the best available scientific data for the purposes of conducting a risk assessment. Approval of this Application will encourage an efficient and internationally competitive food industry.

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.

Lipases also have wide use in the dairy industry, specifically for cheese manufacture. 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 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] Applications of lipases, http://www.au-kbc.org/beta/bioproj2/uses.html

These lipases have a role in the preparation of enzyme modified cheeses (EMC), which is discussed in more detail in the Food Technology Report **(Attachment 4)** and in sections 5.2 and 5.3.

5. Relevant Issues

5.1 Risk assessment

Application A517 seeks approval for the use of lipase from a non-genetically modified enzyme, *M. javanicus* 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. Also there are no nutritional or dietary implications in approval of the enzyme since there will be no or very little residual inactivated enzyme in the final produced foods.

The Safety Assessment Report of lipase sourced from *M. javanicus* (**Attachment 3**) concluded that:

- The source organism is non-pathogenic.
- The enzyme preparation complies with international specifications.
- The evidence from the available safety studies with the enzyme did not indicate any adverse effects.
- 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 *M. javanicus* as a processing aid in food would not raise any public health and safety concerns.

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 alternatives being triacylglycerol lipase, triacylglycerol acylhydrolase and phospholipase. This enzyme is already approved in the Code but it has a number of different sources.

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 source *Mucor javanicus*. The enzyme preparation is a white powder. The enzyme preparation meets the international enzyme specifications in the Food Chemicals Codex, 4th Edition, 1996[3] and the Joint FAO/WHO Expert Committee on Food Additives (JECFA)[4], in the Compendium of Food Additives Specifications, Vol 1 Annex 1, FAO 1992 (Addendum 9, 2001).

There are no dietary or nutritional implications for approval of this enzyme. That is because 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.

5.3 Efficacy and technological justification

Lipases are enzymes that catalyse the cleavage of triglycerides to fatty acids. The Applicant claims lipase sourced from *M. javanicus* hydrolyses medium and long chain fatty acids, preferentially from the 1 and 3 positions of triglycerides. Its specific use and justification for use is to produce specific 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 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 (such as cheese, dips, sauces, dressings, soups, snacks etc). 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.

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

The Food Technology Report **(Attachment 4)** provides more information about the purpose and use of the enzyme.

[3] Food Chemicals Codex, (1996). National Academy of Sciences, Food and Nutrition Board, Committee on Food Chemical 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) (2001). General specifications and considerations for enzyme preparations used in food processing. FAO Food and Nutrition Paper 52, Addendum 9, pp 37-39. (The Code is being updated to include reference to Addendum 12 (2004), in drafting included in the Final Assessment Report for A513 – Octanoic acid as a processing aid).

5.4 Other international regulatory standards

The Applicant states that the enzyme has been confirmed independently as self-affirmed Generally Recognized As Safe (GRAS) 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 permission for 'lipase' in the Food Additives approved list in Japan.

5.5 Issues arising from submissions

One submission to the Initial Assessment Report proposed that *M. jejunicus* as a more recent name be used as the source name with an editorial note indicating that *M. javanicus* is an alternative name. FSANZ did not find any mention of *M. jejunicus* in the literature relating to this organism. As well, no mention was found of this name at all in recent microbiological reference texts so this name will not be used in an Editorial note to the Standard.

5.6 Mucor species nomenclature

A scientific search was made to assess the nomenclature of the source organism and to ensure that *M. javanicus* is an appropriate source name to use for drafting. It was also important to know if there are more recent names for the organism and whether they are the same species. Another name referred to in the scientific literature is *M. circinelloides.*

5.6.1 Research Results

The research involved a search of scientific literature and internet sources.

5.6.1.1 Research results indicating a name change are:

Recent scientific publications:

- Antczak et al. (1997)[5] state 'The lipase of *Mucor javanicus* (now *M. circinelloides*)…'
- Dowd and Sheehan (1999)[6] state '*Mucor circinelloides* (previously *M. javanicus*)…'
- Andrade et al. (2003)[7] published a paper entitled 'Effect of medium components and time of cultivation on chitin production by *Mucor circinelloides* (*Mucor javanicus* IFO 4570) - a factorial study'

all of which support the suggestion of a name change for this species.

[5] Antczak, T., Mrowiec-Bialon, J., Bielecki, S., Jarzebski, A.B., Malinowski, J.J., Lachowski, A.I. and Galas, E. (1997), Thermostability and esterification activity of *Mucor javanicus* lipase entrapped in silica aerogel matrix and in organic solvents. Biotechnology Techniques 11(1): 9-11.

[6] Dowd, C.A. and Sheehan, D. (1999), Variable expression of glutathione S-transferase isoenzymes in the fungus, *Mucor circinelloides*. FEMS Microbiol. Lett. 170(1): 13-17.

[7] Andrade, V.S., Neto, B.B., Fukushima, K. and Campos-Takaki, G. M. (2003), Effect of medium components and time of cultivation on chitin production by *Mucor circinelloides* (*Mucor javanicus* IFO 4570)- A factorial study. Revista Iberoamericana de Micologia 20(3,): 149-153.

In the database of the American Type Culture Collection (http://www.atcc.org/), all occurrences of *Mucor javanicus* are as an alternative name to *Mucor circinelloides*: *Mucor circinelloides* f. *circinelloides* Schipper, teleomorph deposited as *Mucor javanicus* Wehmer, teleomorph.

The CABI Bioscience Index Fungorum (http://www.indexfungorum.org/Index.htm) , the world database of fungal names, records the current name for *Mucor javanicus* Wehmer as *Mucor circinelloides* f. *circinelloides* Tiegh.

The Centraalbureau voor Schimmelcultures, an Institute of the Royal Netherlands Academy of Arts and Sciences (http://www.cbs.knaw.nl) lists *M. javanicus* with the taxonomic information stating "*Mucor javanicus* Wehmer, syn. of *M. circinelloides* f. *circinelloides*". and with any cultures received as *M. javanicus* having their named changed in April 1970.

Doctor Fungus (http://www.doctorfungus.org), a website dedicated to timely dissemination of information about fungal infections, funded by sponsorship from pharmaceutical companies, lists *M. javanicus* as one of a number of obsolete synonyms of *M. circinelloides*.

Research results calling into question a name change are:

UniProt, the universal protein knowledgebase (http://www.ebi.uniprot.org) contains reference to proteins derived from both *M. javanicus* and *M. circinelloides*.

The Deutsche Sammlung von Microorganismen und Zellbulturen GmbH (http://www.dsmz.de.species.sp300215.htm) lists both *M. javanicus* and *M. circinelloides.*

The Research Center for Pathogenic Fungi and Microbial Toxicoses, Japan, list both species as available. However, Andrade et al. (2003), *vide supra*, obtained their strain from that institute.

5.6.2 Conclusion

The situation with the nomenclature of this species is made unclear by continued use of the species name *M. javanicus*. The various nomenclature databases and culture collections searched suggest that the species are named as *M. circinelloides* f. *circinelloides* in most recent references.

FSANZ proposes to refer to the requested name, *M. javanicus,* if the Application is successful, since that is the name used in this Application and on which the safety assessment has been carried out. However, an addition to the Editorial note will be included indicating that the two names are used interchangeably for the same microorganism and *M. javanicus* is an earlier synonym of *M. circinelloides* f. *circinelloides*.

5.7 Risk management

The risk assessment performed for the enzyme lipase, triacylglycerol sourced from *M. javanicus* as a processing aid in food concluded that its use would raise no public health and safety concerns.

Dietary modelling is not required for the use of lipase triacylglycerol sourced from *M. javanicus* since the enzyme is not 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 in food processing and not raise any public health and safety concerns is to regulate them as permitted enzymes in Standard 1.3.3 – Processing Aids. 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 will be listed. This proposed drafting is included in **Attachment 1**.

FSANZ is currently undertaking a review of enzyme permissions within Standard 1.3.3 – Proposal P276 – Review of Processing Aids (Enzymes) where the current Editorial note will be reviewed to ensure the various statements concerning alternative names for microorganisms are correct.

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 *M. javanicus* as a processing aid.

Option 2. Approve the use of lipase, triacylglycerol sourced from *M. javanicus* 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, vegetarian consumers and those consumers preferring Kosher certification of 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 sourced from *M. javanicus* 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 18 February until 31 March 2004. Three submissions were received of which two supported the Application and one reserved comment until the Draft Assessment). **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;
- nomenclature of the source organism, that *M. javanicus* is also known as the more recent name of *M. circinelloides* f. *circinelloides;*
- 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 *Mucor javanicus* is unlikely to have a significant effect on international trade as most countries do not regulate enzymes as processing aids as in Australia and New Zealand. Also since it is a processing aid there is no requirement to label final foods for the presence of the enzyme. The enzyme preparations are consistent with the international specifications for food enzymes of the Food Chemicals Codex (4th Edition, 1996) and JECFA so was determined that there was no need to notify the WTO under either the Sanitary and Phytosanitary (SPS) or the Technical Barriers to Trade (TBT) Agreements.

9. The Decision

Approval is proposed for the enzyme, lipase, triacylglycerol (EC [3.1.1.3]) from a new microbiological source, namely the fungus *Mucor javanicus*. Permission is given by adding this approval 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 of the Code, thereby giving approval for the use of lipase, triacylglycerol sourced from *M. javanicus* as a processing aid is recommended for the following reasons.

- 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.

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

- The source organism, *M. javanicus* is a well understood organism (filamentous fungi) that is considered non-pathogenic.

- 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, triacylglycerol sourced from *M. javanicus* 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 inserting into the* Table to clause 17 –

Lipase, triacylglycerol EC [3.1.1.3]	*Mucor javanicus*

[2] ***Standard 1.3.3*** *of the Australia New Zealand Food Standards Code is varied by inserting into the* Editorial Note to clause 17 –

Mucor javanicus is the former name for *Mucor circinelloides* f. *circinelloides*

Attachment 2

Summary of public submissions

Round One

#	Submitter Organisation	Name
1	Food Technology Association Vic	David Gill
2	New Zealand Food Safety Authority	Carole Inkster
3	Australian Food and Grocery Council	Tony Downer

Submitter	Position	Comments
Food Technology Association Vic	Agrees, supports option 2	It supports the application.
New Zealand Food Safety Authority	No position at this stage, may do so at Draft Assessment	It may provide comments at the Draft Assessment stage.
Australian Food and Grocery Council	Agrees, supports the Application	Other comments are: • The AFGC considers it unlikely that FSANZ will determine that the lipase from *Mucor javanicus* is unsafe. • The use of the enzyme is technologically justified. • They suggest the more recent name (*Mucor jejunicus*) be used in the Code, with appropriate clarification of alternative names (*Mucor javanicus*, the name in the Application) in the editorial note as is currently done.

Attachment 3

Safety assessment report

Summary and Conclusion

Application A517 seeks approval for the use of lipase from a non-genetically modified *M. javanicus* 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 sourced from *M. javanicus* concluded that:

- The source organism is non-pathogenic.
- The enzyme preparation complies with international specifications.
- The evidence from the available safety studies with the enzyme did not indicate any adverse effects.
- 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 *M. javanicus* as a processing aid in food would not raise any public health and safety concerns.

1. Introduction

Application A517 seeks approval for the use of lipase from a non-genetically modified *M. javanicus* as a processing aid. The microbial source is the fungi *M. javanicus* (also called *M. circinelloides*).

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.

Numerous studies relevant for the safety assessment were submitted in support of this Application. These were: a) a pathogenicity study of *M. javanicus* in mice, b) acute toxicity studies in mice and rats c) short-term oral toxicity studies in mice and rats, d) a sub-chronic oral toxicity study in rats, e) teratology studies in mice and rats, f) a reverse mutation test in bacteria, g) a chromosome aberration test, and h) some clinical studies with patients with gastrointestinal disturbances.

2. The source (production) organism – *Mucor javanicus*

The source organism, *Mucor javanicus* is generally considered not to produce mycotoxins. It is an occasional opportunistic human pathogen (Ellis, 1994; Rippon, 1988), being a relatively rare cause of acute and rapidly developing zygomycoses in debilitated patients. Susceptible groups include acidotic diabetics, malnourished children, severely burned patients, people with leukaemia, lymphoma, and AIDS and those undergoing immunosuppressive therapy.

Infection usually occurs via the respiratory tract or by oral-mucosal contact with contaminated material (Bossche, 1990).

Mucor species are commonly found in foods (Tsai *et al*, 1993). *M. circinelloides* has been described as a component of the mould starter cultures used for the commercial preparation of sufu, a Chinese soybean cheese obtained by solid-state fungal fermentation of tofu (Han *et al*, 2004).

Pathogenicity study of *Mucor javanicus* in mice (Mizutani, 2000)

Test material	viable cells of *Mucor javanicus*; lot no AYL
Vehicle material	0.01% Tween 80 in saline
Test Species	S1c:ICR female mice (10 animals/dose)
Dose	0, 6.3×10^4, 6.3×10^5, 6.3×10^6 cells/mice (gavage)
GLP/guidelines	not reported, quality assurance reported.

Groups of 10 female mice received single doses of a spore suspension of *Mucor javanicus* administered by gavage. The animals were observed for 14 days post-dose. Body weights were recorded at day 0, 1, 3, 5, 7, 10, 14. At day 15, the animals were sacrificed and necropsy was performed. Brain, liver and kidneys were assessed for histopathology and viable fungi. No clinical signs and mortality was observed. Necropsy revealed no treatment related effects and no viable fungi were detected in brain, liver and kidneys.

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, 2003).

Table 1: Complete specification of lipase sourced from *Mucor Javanicus*

Criteria	Specification
Esterase activity (U/g)	5220 (+/- 10%)
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 *Mucor javanicus*

The safety studies were conducted with the enzyme lipase M-AP10. This is the marketed name of lipase sourced from *M. javanicus* produced by Amano Pharmaceuticals. Therefore, these studies were considered relevant for the safety assessment of lipase sourced from *M. javanicus*. With the exception of the genotoxicity studies, the studies were not presented in a currently acceptable way. A summary of these studies is presented below. While the studies were old, and not according to international standards, the studies can give some evidence regarding the safety of lipase sourced from *M. javanicus*.

4.1 Acute toxicity studies

Acute oral toxicity study in mice (Fukuda, date unknown)

Test material	Lipase M-AP10
Vehicle material	water suspension (0.5% CMC)
Test Species	dd strain male mice (3 animals/dose for first, 10 animals/dose for second experiment)
Dose	0, 1, 5, 7, 10 g/kg bw first experiment, 5, 10, 20 g/kg bw second experiment
GLP/guidelines	none

Groups of 3 or 10 male mice per dose received single doses of lipase M-AP10 orally and were observed for mortality for 7 days post-dose. Bodyweights were measured prior to dosing, at day 1, 2, and 7. No mortality was observed and bodyweights revealed no treatment-related effects.

Acute oral toxicity study in mice (Migami, date unknown[a])

Test material	Lipase M-AP10
Vehicle material	distilled water
Test Species	JCL-ICR mice (5 animals/sex/dose)
Dose	0, 5, 10, 20 g/kg bw
GLP/guidelines	none

Groups of 5 female and 5 male mice per dose received single doses of lipase M-AP10 orally and were observed for mortality for 7 days post-dose. Bodyweights were measured prior to dosing, at day 1, 2, and 7. At day 7, necropsy was performed and the weight of liver kidney and spleen was determined. No mortality was observed. Bodyweights and necropsy revealed no treatment-related effects.

Acute oral toxicity study in rats (Migami, date unknown[b])

Test material	Lipase M-AP10
Vehicle material	distilled water
Test Species	Wistar rats (5 animals/sex/dose)
Dose	0, 5, 10, 20 g/kg bw
GLP/guidelines	none

Groups of 5 female and 5 male rats per dose received single doses of lipase M-AP10 orally and were observed for mortality for 7 days post-dose. Body weights were measured prior to dosing, at day 1, 2, and 7. At day 7, necropsy was performed and the weight of liver kidney and spleen was determined. No mortality was observed. Bodyweights and necropsy revealed no treatment-related effects.

4.2 Short-term toxicity

Short-term oral toxicity study in mice (Migami, date unknown[c])

Test material	Lipase M-AP10
Control and vehicle material	distilled water
Test Species	JCL-ICR mice (5 animals/sex/dose)
Dose	0, 5, 10, 20 g/kg bw for 30 days
GLP/guidelines	none

Study conduct

Groups of mice (5/sex/group) were treated with lipase by gavage at 0, 0.1, 2 or 5 g/kg bw per day for 30 days.

Bodyweight was recorded. At the end of the study, all animals were sacrificed and necroscopy performed (selected organ weights, liver, kidney, spleen, lung and heart) and histopathology on selected organs was performed.

Results

No mortality was observed during treatment. No dose-related effects were observed on bodyweight increase (only a graph was provided). Data from one male of the middle dose group was not provided; therefore it is unknown whether this animal died due to treatment. No dose-related effects on organ weights were observed. Data on histopathology were not provided.

Short-term oral toxicity study in mice (Migami, date unknown[d])

Test material	Lipase M-AP10
Control and vehicle material	distilled water
Test Species	Wistar rats (5 animals/sex/dose)
Dose	0, 5, 10, 20 g/kg bw for 30 days
GLP/guidelines	none

Study conduct

Groups of rats (5/sex/dose) were treated with lipase by gavage at 0, 0.1, 2 or 5 g/kg bw per day for 30 days.

Bodyweight was recorded. At the end of the study, all animals were sacrificed and necroscopy performed (selected organ weights, liver, kidney, spleen, lung and heart) and histopathology on selected organs was performed.

Results

No mortality was observed during treatment. No dose-related effects were observed on bodyweight increase (only a graph was provided). No dose-related effects on organ weights were observed. Data on histopathology were not provided.

Sub-chronic oral toxicity study in rats (Migami, date unknown[e])

Test material	Lipase M-AP10
Control and vehicle material	distilled water
Test Species	female Wistar rats (unknown number of animals per dose)
Dose	0, 5, 10, 20 g/kg bw for 3 or 6 months
GLP/guidelines	none

Study conduct

Groups of female rats (number per dose unknown) were treated with lipase by gavage at 0, 0.1, 2 or 5 g/kg bw per day for three or six months.

Bodyweight was recorded. In the group of animals treated for three months some haematology parameters (leucocyte index, erythrocyte, haematocrit, haemoglobin) were measured of some animals. After six months of treatment some haematology parameters (leucocyte index, erythrocyte, haematocrit, haemoglobin), and clinical chemical parameters (albumin globulin, albumin globulin ratio, GOT, GPT) were measured in some animals. At the end of the study, all animals were sacrificed and necroscopy performed (selected organ weights, liver, kidney, spleen, lung and heart) and histopathology on selected organs was performed (data not shown).

Results

Data on mortality was not provided. Graphs with bodyweight changes (190 days of male rats) did not indicate an effect on bodyweight, however individual data were not shown.

In the three-month study, data on haematology were not reliable (individual data was available, however many animals did not have results for various parameters). Organ weights were available from only 5, 2, 4, 5 animals of the 0, 0.1, 2 and 5 g/kg bw per day group. Therefore, these data are also not reliable.

In the six-month study, data on haematology and clinical chemistry were not reliable (individual data was available, however many animals did not have results for various parameters). There were no treatment related effects on organ weights, however it was unclear whether individual data from all rats was available. Data on histopathology were not provided.

While this study, did not meet currently acceptable standards, it did not indicate any adverse effects.

4.3 Teratology

Teratology study in mice and rats (Migami, 1966; Migami 1967)

Test material	Lipase M-AP10
Control and vehicle material	distilled water
Test Species	JCL-ICR female pregnant mice (15 animals/dose), Wistar female pregnant rats (10-11 animals/dose)
Dose	0, 5, 10, 20 g/kg bw on day 7-13 (mice) or day 8-14 (rats) of pregnancy
GLP/guidelines	none

Study conduct

Groups of female mice (15/dose) were treated with lipase by gavage at 0, 0.1, 2 or 5 g/kg bw per day from day 7 to 13 of pregnancy. The animals were sacrificed on day 18. Groups of female rats (10-11/dose) were treated with lipase by gavage at 0, 0.1, 2 or 5 g/kg bw per day from day 8 to 14 of pregnancy. The animals were sacrificed on day 20.

Bodyweight, uterus weight, numbers of embryo, live foetus, male/female ratio, body weight of embryo, ano-genital distance, death embryo, organ weight, bones and histological screening was recorded. The study design was not according to international guidelines.

Results

No mortality was observed. No treatment-related effects were observed on maternal or foetal parameters measured. Only limited parameters were recorded, e.g. no observation of early and late resorptions and malformations was made.

While the study was not performed according to currently acceptable standards, the study did not indicate any adverse effects on teratology due to lipase from *M. javanicus*.

4.4 Genotoxicity studies

Reverse mutation test in bacteria (Watanabe, 1999)

Test article

The test article, raw Lipase M powder (Lot No LM-N51-002, 22,800 u/g of lipase activity) was used. Lipase M is produced by *M. javanicus*.

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 OECD guideline 471.

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 *M. javanicus* did not exhibit mutagenic activity under the conditions of the test.

Chromosome aberration test in cultured Chinese hamster cells (Nakagawa, 2004)

Test article

The test article, Lipase M, lot no. LM-N56-002 was used.

Study design

The potential of lipase M 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. The study design was in accordance with OECD guideline 473.

Tests were carried out in the presence and absence of S9 metabolic activation, over a broad range of doses. In a dose finding study in both the absence or presence of S9, the cells were treated for six hours and the harvest time was 18 hours after treatment stopped. One continuous treatment study for 24 hr was conducted in the absence of S9. The concentrations inducing 50% growth inhibition were estimated to be over 5000 μg/ml (6 hour treatment, in absence of S9 and in the 24 hour test) and 2377 μg/ml (metabolic activation test). Based on these results, the treatment levels in the main studies were 1250, 2500, and 5000 μg/ml in the absence of S9 using a continuous treatment until harvest at 24 hours; 1250, 2500 and 5000 μg/ml in the absence of metabolic activation for six hours; and 313, 625, 1250, 2500, and 5000 μg/ml in the presence of metabolic activation for six hours.

Test	*Test material*	*Concentration*	*Test object*	*Result*
chromosome aberration (*In vitro*)	Lipase M	0, 1250, 2500, and 5000 µg/ml continuous treatment 0, 1250, 2500, 5000 µg/plate, without S9 mix, for 6 hours 0, 313, 625, 1250, 2500, 5000 µg/plate, with S9 mix, for 6 hours	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.

In conclusion, lipase M did not have the ability to induce chromosomal aberration under the conditions of the test.

4.5 Clinical studies

The Applicant submitted five clinical studies to support the Application. Three studies did not report the dose levels used in their studies and could therefore be not used for the safety assessment (Kishikawa, date unknown, Author unknown, date unknown, Takasaki H, date unknown). Two studies did report the doses used (Mori, date unknown, Ueno Y, date unknown).

In the study by Mori (date unknown) 30 patients with acute or chronic gastrointestinal diseases were given 2 capsules (40 mg/capsule) of Lipase M-AP10 after each meal (6 capsules daily; 240 mg lipase/day) for one to three weeks. According to the study author treatment with Lipase M-AP10 improved the gastrointestinal symptoms in 25 of 30 patients, and side effects were not observed.

In the study by Ueno (date unknown) 31 patients with gastrointestinal diseases were given three or six capsules (170 mg/capsule) of Lipase M-AP10 after each meal for one to four weeks. According to the study author treatment with Lipase M-AP10 improved the gastrointestinal symptoms in 24 of the 31 patients, and side effects were not observed.

These studies have limited value for the safety assessment of lipase from *Mucor javanicus.* It was not reported how adverse side effects were monitored. However, at high doses (up to 3 g/day) symptoms such as anorexia, vomiting, fullness, fatigue, nausea, diarrhoea, were decreased.

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Attachment 4

Food technology report

A517 – LIPASE FROM *MUCOR JAVANICUS* AS A PROCESSING AID (ENZYME)

Introduction

FSANZ received an application from Biocatalysts Ltd to amend the Code to approve a new source, the filamentous fungi *M. javanicus*, 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. The enzyme is already approved in the Code but with a number of other sources, not including *M. javanicus*. An alternative name of the organism is *M. circinelloides*.

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.

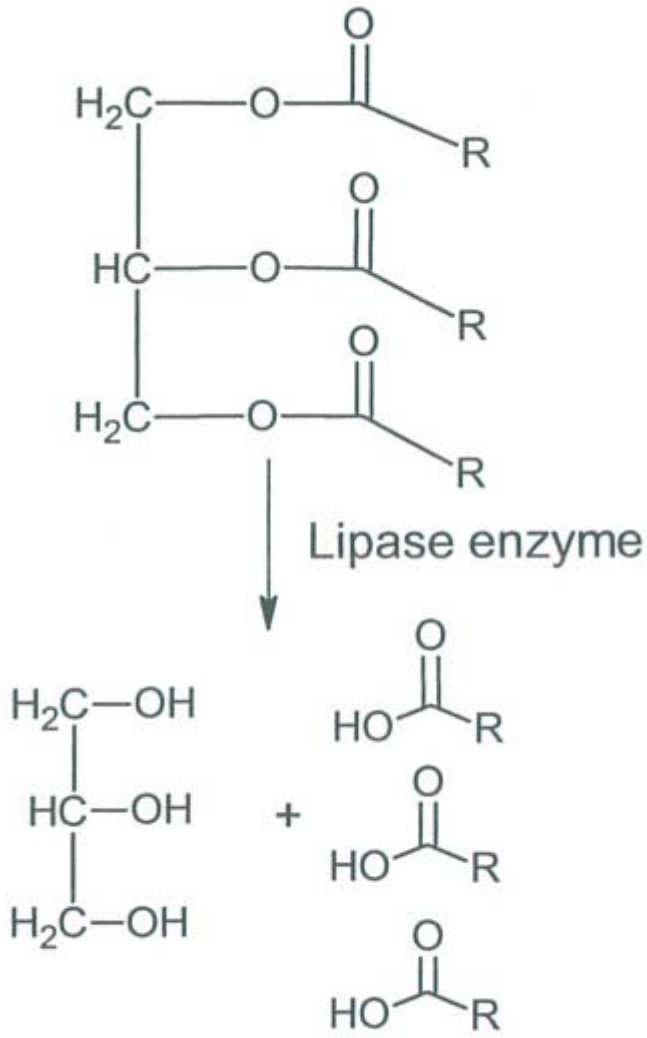
The enzyme for this Application is from a microbial source (the filamentous fungi *M. javanicus*) rather than an animal source.

The enzyme preparation is a white powder with pH stability between 4 and 8 and optimum pH between 6-7. 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 (with some cleavage of position 2 fatty acids) so it is able to cleave medium and long 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 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 *M. javanicus* hydrolyses medium and long chain fatty acids from the number 1 and 3 positions of triglycerides (with limited activity at position 2). It specific use and justification for use is to produce specific cheese flavours.

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[1]. 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 (such as cheese, dips, sauces, dressings, soups, snacks etc). Bland flavoured immature cheese (processed 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 microorganism *M. javanicus*. 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).

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 *M. javanicus* 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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