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

7-05
5 October 2005

FINAL ASSESSMENT REPORT

APPLICATION A516

LIPASE FROM *CANDIDA RUGOSA* AS A PROCESSING AID (ENZYME)

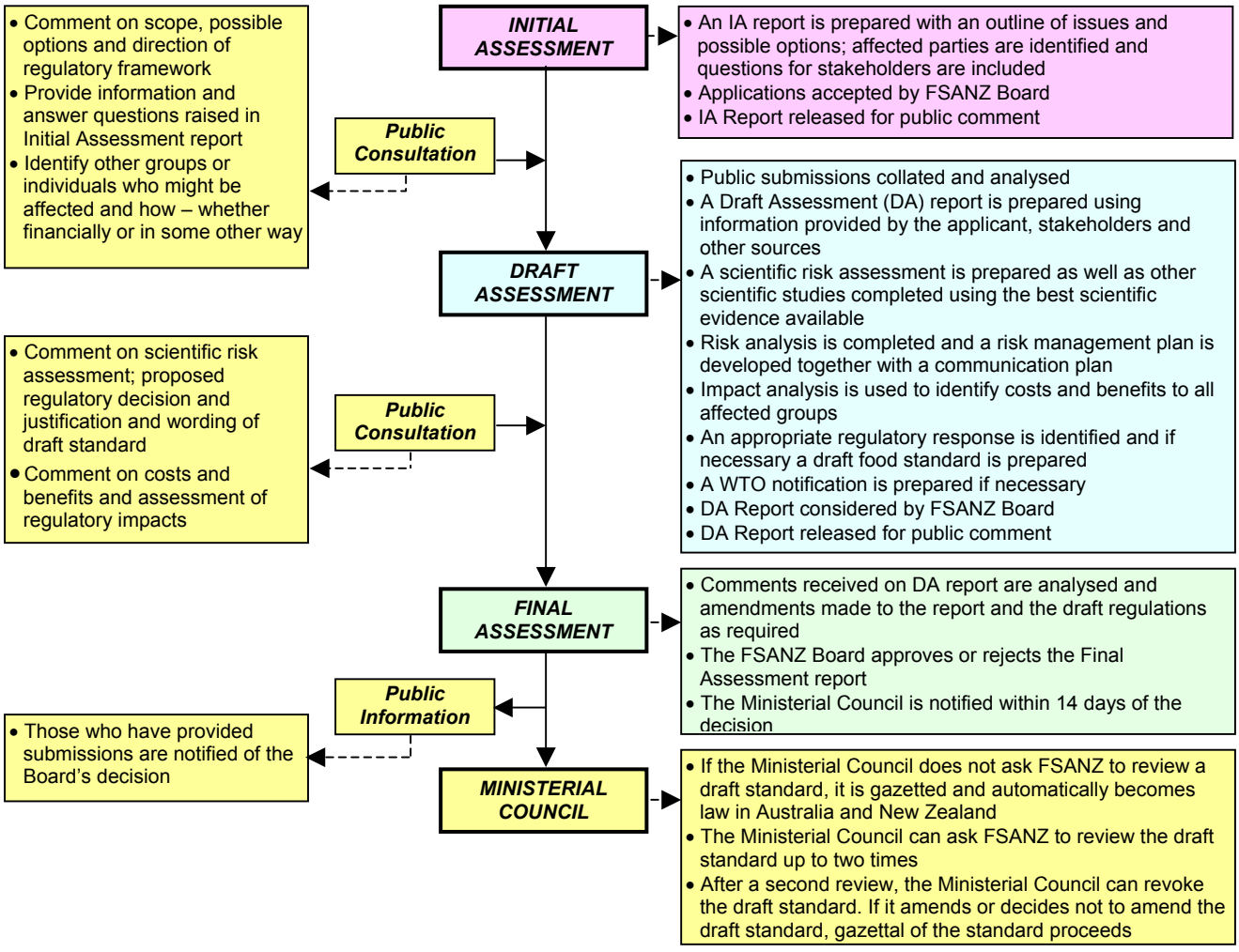
FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

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.



Final Assessment Stage

FSANZ has now completed two stages of the assessment process and held two rounds of public consultation as part of its assessment of this Application. This Final Assessment Report and its recommendations have been approved by the FSANZ Board and notified to the Ministerial Council.

If the Ministerial Council does not request FSANZ to review the draft amendments to the Code, an amendment to the Code is published in the *Commonwealth Gazette* and the *New Zealand Gazette* and adopted by reference and without amendment under Australian State and Territory food law.

In New Zealand, the New Zealand Minister of Health gazettes the food standard under the New Zealand Food Act. Following gazettal, the standard takes effect 28 days later.

Further Information

Further information on this Application and the assessment process should be addressed to the FSANZ Standards Management Officer at one of the following addresses:

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Assessment reports are available for viewing and downloading from the FSANZ website www.foodstandards.gov.au or alternatively paper copies of reports can be requested from FSANZ's Information Officer at info@foodstandards.gov.au including other general inquiries and requests for information.

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Executive Summary and Statement of Reasons

FSANZ received an Application on 6 November 2003, from Biocatalysts Ltd (Wales, UK), 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 enzyme is derived from a new microbial source, the yeast *Candida rugosa*. The enzyme is not sourced from a genetically modified organism.

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 the yeast *C. rugosa* 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 *C. rugosa*.

At Initial Assessment the Applicant requested to list *C. cylindracea* as the source organism and *C. rugosa* was suggested as an alternative name for the organism. The taxonomic nomenclature situation regarding the source organisms is confusing but recent expert microbiological advice FSANZ has received is that the two sources are not identical and should be considered separate species. The Applicant subsequently altered their request for the source organism to *C. rugosa*.

The new enzyme has broad activity for hydrolysing triglycerides to release fatty acids from all three triglyceride positions, in soft and hard fats. It is claimed to have a high affinity for short chain fatty acids, in particular C4 (butyric acid) to produce desirable flavours for processed cheese.

The enzyme is used as a processing aid only, and is not expected in most usage situations 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 *C. rugosa* 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 1250 mg/kg bw per day.
- 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, triacylglycerol from *C. rugosa* 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. 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 food in Japan.

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

FSANZ sought public comment on the Initial Assessment Report from 18 February to 31 March 2004. Three submissions were received of which two supported the Application and one reserved comment until the Draft Assessment.

FSANZ sought public comment on the Draft Assessment Report from 25 May till 6 July 2005. Five submissions were received, four which supported the approval of the enzyme, and one raised an issue which has been addressed.

FSANZ Decision

Approval is given for the enzyme, lipase, triacylglycerol, from a new microbiological source, namely the yeast *Candida rugosa*. 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 of the Code, giving approval for the use of the lipase, triacylglycerol enzyme sourced from *C. rugosa* as a processing aid, is recommended 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, *C. rugosa* is well known and considered non-pathogenic and non-toxigenic.
- 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 *C. rugosa* 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 Biocatalysts Ltd (Wales, UK), 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, the yeast *C. rugosa*. The enzyme is not sourced from a genetically modified organism.

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

The Application originally listed *C. cylindracea* as the source organism. The Initial Assessment Report stated that a more recent common name for the organism is *Candida rugosa.* However, during the Draft Assessment this statement was investigated further and conflicting information was received. FSANZ received advice from a renowned mycology expert that the two yeasts can both be considered as valid species and the names should not be used interchangeably. The Applicant subsequently altered their request for the source organism to *C. rugosa*.

The main function for this source of the enzyme is to hydrolyse triglycerides to release fatty acids from all three triglyceride positions, in soft and hard fats. It is claimed to have a high affinity for short chain fatty acids, in particular C4 (butyric acid) to produce 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 *C. rugosa*. *C. rugosa* 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 being assessed at Draft Assessment. This Application is A517, which is seeking approval for another source for the enzyme, lipase triacylglycerol, sourced from *Mucor javanicus*. This lipase enzyme from the different source produces a different flavour profile from that of this Application, since the enzyme has slightly different activity towards triglycerides.

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 *Candida rugosa* 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.

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, 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).

[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

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

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

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.

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

a) a pathogenicity study of *C. rugosa* 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;
e) a mutation test in mouse lymphoma cells; and
f) a chromosomal aberration test in cultured Chinese hamster cells.

The safety assessment of lipase from *C. rugosa* 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 1250 mg/kg bw per day.
- 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 *C. rugosa* 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. Its common name is lipase, with other alternatives being triacylglycerol lipase, triacylglycerol acylhydrolase and phospholipase. As mentioned earlier in the report there is already approval for this enzyme in the Code but with 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 yeast source *C. rugosa*. 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 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)[4].

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, which is 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 *C. rugosa* is a broad spectrum lipase which attacks all three triglyceride positions, in both hard and soft fats. However, it has more specific activity for short chain fatty acids, in particular C4 (butyric acid). Its specific use and justification for use is to produce cheese flavours. Typical cheese characteristic flavours include short chain fatty acids of the C4 and C5 length such as butyric acid and isovaleric acid.

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 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 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.

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

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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) (2001). General specifications and considerations for enzyme preparations used in food processing. FAO Food and Nutrition Paper 52, Addendum 9, pp37-39. (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 US Food and Drug Administration (FDA) has not questioned the self-affirmed Generally Recognized As Safe (GRAS) status of the enzyme from this source. The FDA GRAS notice is GRN 000081, 7/2/02[5]. The GRAS notice is for the source organism *C. rugosa.* The Applicant also states the enzyme is approved for food use and is listed on the Food Additive list in Japan. The enzyme has been certified as Kosher by the New York Orthodox Union and Manchester Beth Din.

5.5 Issues addressed from submissions

5.5.1 Name of the source organism

One submission to the Initial Assessment Report proposed that *C. rugosa* be used as the source name with an editorial note provided that *C. cylindracea* is an alternative. FSANZ performed a microbiological assessment of the two names to review the organism nomenclature. Recent expert advice received by FSANZ is that these two yeasts can both be considered as valid species and the names should not be used interchangeably (**Attachment 5** – Microbiological Assessment Report). Therefore the suggested Editorial note is not recommended.

The Applicant subsequently amended their Application seeking the approval for lipase sourced from *C. rugosa*, not *C. cylindracea*, so if successful approval will be given only for *C. rugosa* as the source.

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.

5.5.2 Breadth of the permitted use 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'.

5.5.2.1 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.

[5] US Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Food Additive Safety, GRAS Notice No. GRN 000081, http://www.cfsan.fda.gov/~rdb/opa-g081.html

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.

5.6 Risk management

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

There are no dietary modelling issues with the use of lipase, triacylglycerol sourced from *C. rugosa* 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 would 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 need to be listed. This proposed drafting is listed in **Attachment 1**.

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 *C. rugosa* as a processing aid.

Option 2. **Approve** the use of lipase, triacylglycerol sourced from *C. rugosa* 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 Commonwealth, 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 *C. rugosa* 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 till 31 March 2004. Three submissions were received of which two supported the Application and one reserved comment until the Draft Assessment. Public comment on the Draft Assessment Report was sought from 25 May till 6 July 2005. Five submissions were received of which four supported the approval of the enzyme. One submission did not state a direct position but raised an issue that has been addressed in an earlier section (section 5.5). **Attachment 2** summarises the submissions received during this first and second round of public comment.

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 *C. rugosa* 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 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

The Final Assessment Report concludes that the approval of the use of lipase, triacylglycerol sourced from *C. rugosa* as a processing aid is technologically justified and does not pose a risk to public health and safety.

Approval is given for the enzyme, lipase, triacylglycerol, from a new microbiological source, namely the yeast *C. rugosa*. 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 is recommended 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.
- The source organism, *C. rugosa* is well known and is considered non-pathogenic and non-toxigenic.
- 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 triacylglycerol sourced from *C. rugosa* 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
5. Microbiological assessment 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]	*Candida rugosa*

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 *Candida cylindracea* is unsafe. • The use of the enzyme is technologically justified. • The AFGC suggests the more recent name (*Candida rugosa*) be used in the Code, with appropriate clarification of alternative names (*Candida cylindracea*, the name used in the Application) in the editorial note as is currently done.

Round Two

#	Submitter Organisation	Name
1	Victoria Department of Human Services	Victor Di Paola
2	New Zealand Food Safety Authority	Carole Inkster
3	New South Wales Food Authority	Kelly Boulton
4	Queensland Health	Gary Bielby
5	Australian Food and Grocery Council	Kim Leighton

Submitter	Position	Comments
Victoria Department of Human Services	Supports	It supports option 2, to approve use of the enzyme.
New Zealand Food Safety Authority	Supports	It supports option 2, to approve use of the enzyme.
New South Wales Food Authority	Did not state a position, however seem to have concerns	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.
Queensland Health	Supports	It supports option 2, to approve the use of the enzyme.
Australian Food and Grocery Council	Supports	It supports option 2, to approve the use of the enzyme.

Attachment 3

Safety assessment report

Application A516 – Lipase sourced from *Candida rugosa*

Summary and Conclusion

Application A516 seeks approval for the use of lipase triacylglycerol from a non-genetically modified *Candida rugosa* 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 *C. rugosa* 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 1250 mg/kg bw per day.
- 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 *C. rugosa* as a processing aid in food would not raise any public health and safety concerns.

1 Introduction

Application A516 seeks approval for the use of lipase triacylglycerol from a non-genetically modified *C. cylindracea* 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.

Six studies relevant for the safety assessment were submitted in support of this application. These were: a) a pathogenicity study of *C. rugosa* 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, e) a mutation test in mouse lymphoma cells, and f) a chromosomal aberration test in cultured Chinese hamster cells.

The studies above have also been published as a review article on the safety of lipase produced from *Candida rugosa* (Flood and Kondo, 2001).

2 The source (production) organism – *Candida rugosa*

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

In application A516 the approval is sought for the use of lipase from a non-genetically modified *C. rugosa* as a processing aid.

One pathogenicity study on *C. rugosa* was submitted that is summarised below. A search in the literature revealed a few studies indicating that *C. rugosa* might be mildly pathogenic in certain circumstances. In immune-suppressed mice *C. rugosa* isolated from bovine mastitis secretion produced moderate pathogenicity (Jensen and Aalbaek, 1994). Furthermore there is some evidence that *C. rugosa* is associated with pathogenicity in humans that had invasive medical procedures (Colombo *et al*, 2003). *C. rugosa* is an animal pathogen and causes candidiasis in cattle and may cause mastitis. However, only a few cases of fungaemias in humans have been reported. The common risk factor was burn wounds and surgical nystatin prophylaxis (Krcmery and Barnes, 2002).

Pathogenicity study on *Candida rugosa* in mice (Anon, 1992)

Test material	viable cells of *Candida rugosa*; lot no AYL
Vehicle material	saline
Test Species	S1c:ICR female mice (5-10 animals/dose)
Dose	0, 1.5×10^3, 1.5×10^5, 1.5×10^7 cells/mice (intravenously administration)
GLP/guidelines	not reported.

Groups of 5-10 female mice received single doses of a spore suspension of *C. rugosa* administered intravenously. The animals were observed for 14 days post-dose. At day 15 the animals were sacrificed and necropsy was performed. Brain, liver and kidneys were assessed for histopathology and living yeasts. No clinical signs and mortality was observed. Necropsy revealed no treatment related effects and no living yeasts were detected in the brain, liver and kidneys.

C. rugosa does not appear to be pathogenic in healthy mice under normal conditions. Furthermore, the exposure through the use of *C. rugosa* as source for the production of lipase would be negligible. 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).

Table 1. Complete specification of lipase sourced from *Candida rugosa*

Criteria	Specification
Lipase activity (U/g)	115,000 (+/- 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

Criteria	Specification
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 *Candida rugosa*

4.1 Acute study

Oral acute toxicity tests in mice and rats (Murata, 1987)

Test material	Lipase AY produced by C*andida rugosa*, activity 79,700 units/g
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, 1.25, 2.50, 5.0 g/kg bw
GLP/guidelines	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 (Matsubara, 1995)

Test material	Lipase AY produced by *Candida rugosa*, lot no. 61218TM, 79,700 U/mg
Control and vehicle material	Sterile water
Test Species	SPF Crj : CD (SD) rats 10 males and females per test dose; administration by gavage
Dose	0, 625, 1250, 2500 mg lipase/kg bw per day
GLP/guidelines	signed GLP and quality assurance statement; Guideline not specified

Study conduct

Groups of rats (10/sex/group) were treated with lipase by gavage at 0, 625, 1250 or 2500 mg/kg bw per day for 13 weeks. In two additional groups (10/sex/group) after 13 weeks of treatment at 0 and 2500 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 5 animals/sex/group in week 11-13 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.

Since potassium levels were increased in the urine in the main study, a retest was performed in male rats (6/group) in a control and 2500 mg/kg bw per day group. The animals were treated for 90 days and then a recovery period of 60 days was included. Clinical observations were recorded daily. Bodyweight and food consumption were recorded twice weekly. Urinalysis was performed before treatment, at 30, 60 and 90 days of treatment and 30 and 60 days after treatment had ceased.

Results

No mortality was observed during treatment of lipase in the main study. Occasional injury of the upper jaw was found in almost all groups and ascribed to the stainless steel inner lid. In the retest study starvation, resulting from deformity of the upper jaw caused by the stainless steel inner lid of the powdery feed led to the death of one male rat at 2500 mg/kg bw.

No dose related effects were observed on body weight, food consumption and ophthalmology. Dose related increases in urine potassium levels were observed after 90 days of treatment in both males and females, reaching statistical significance at 1250 mg/kg bw per day (see table 2). Urine chloride levels were increased statistically significantly in males at 2500 mg/kg bw/day and in females at 1250 and 2500 mg/kg bw/day. At the end of the recovery period statistically significant increases of urinary potassium levels were seen in both sexes at 2500 mg/kg bw/day.

Table 2: various plasma and urine parameters in 90-day study in rats

	Main study (treated 90 days)				Recovery (30 days	
	0	625	1250	2500	0	2500
Males						
plasma [K^+]	4.71	4.73	4.69	4.52	4.70	4.78
urinary [K^+]	182	227	279*	569***	195	390**
urinary [Cl^-]	44.5	44.4	59.7	136.4*	48.3	83.3
urine flow	18.6	16.6	19.8	16.7	19.6	17.1
Females						
plasma [K^+]	4.32	4.26	4.12	4.25	4.12	4.41**
urinary [K^+]	221	357	451**	527**	211	585***
urinary [Cl^-]	49.4	58.6	101.2*	95.4*	48.9	82.7
urine flow	14.7	17.4	15.0	17.4	17.6	18.7

In the retest, urinary potassium levels were measured 0-4 h after treatment and 4-22 h after treatment. Potassium and chloride excretion was increased 0-4 h after treatment, however no statistically significant differences between control and treatment were observed 4-22 h after gavage treatment. In the recovery period, no statistically significant differences in urinalysis were observed, however there was a tendency of higher potassium excretion.

Plasma potassium levels were slightly decreased in males at 2500 mg/kg bw/day compared to controls, however statistical significance was not reached (see table 2). 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 potassium concentrations by the adding of inorganic salt during production of the test substance; the crude test substance contained 2.85% K^+. This potassium load could explain part of the increased potassium concentration in urine; however there was still increased potassium excretion four weeks after treatment was ended.

High potassium excretion can result in hyperkalaemia (low potassium levels in the plasma). Hyperkalaemia can cause rapid and irregular heart rhythm, muscle weakness and irritability, occasional paralysis, nausea and vomiting, diarrhoea and low muscle tone in the gut, and it has been reported to predispose to hypertension. No abnormal findings were seen in the various organs that would give an indication of hyperkalaemia, however animals were only treated for 90 days. Long-term impact of high potassium concentrations in urine on various organs has not been studied. Since both the long term impact have not been studied and potassium concentrations in urine were elevated four weeks after treatment was ended the increased potassium levels in urine are a relevant adverse effect.

The NOEL was 1250 mg/kg bw per day, based on the increased potassium concentrations in urine at 2500 mg/kg bw per day, which remained after a recovery period of four weeks.

4.3 Genotoxicity studies

Reverse mutation test in bacteria (Anon, 1996)

Test article

The test article, raw Lipase AY powder (Lot No LAY-N49-001, 153,000 u/g of lipase activity) was used. Lipase AY is produced by *C. rugosa*.

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 *C. rugosa* did not exhibit mutagenic activity under the conditions of the test.

Mutation assay using L5178Y mouse lymphoma cells (Tanaka, 1996)

Test article

The test article, Lipase AY, lot number LAY-N49-001 was used. The purity was 135,000 units/g lipase activity. Lipase AY is produced by *C. rugosa*.

Study design

Lipase AY was examined for mutagenic activity using the mouse lymphoma forward mutation assay. The mouse lymphoma forward mutation assay evaluated the test article's mutagenic potential in a specific locus mutation assay using mammalian cells in culture. The objective of this study was to evaluate the ability of lipase AY to induce forward mutations at the thymidine kinase (TK) locus in L5178Y TK+/- mouse lymphoma cells as assayed by colony growth in the presence of 5-trifluorothymidine (TFT). Positive controls were treated with the known mutagens cyclophosphamide and methylmethane sulfonate.

A preliminary cytotoxicity experiment was performed to establish an appropriate concentration range for the mutation experiment with and without metabolic activation. Tests were carried out in the presence and absence of S9 metabolic activation, over a broad range of doses. In the first experiment, both in the absence and presence of S9, the cells were treated for 3 hr. Since in toxicity experiments effects on the survival were absent, the treatment levels in the main studies were 1300, 1800, 2500, 3500, and 5000 µg/ml both in the absence and presence of metabolic activation. As negative results were obtained, a second experiment was performed using the same treatment conditions.

Test	*Test material*	*Concentration*	*Test object*	*Result*
Reverse mutation (*In vitro*)	Lipase AY	First and second test: 0, 1300, 1800, 2500, 3500, 5000 µg/plate, with and without S9 mix	mouse lymphoma L5178Y cell line	-ve

Results and conclusion

Treatment did not produce biologically or statistically significant increases in the frequency of mutations at any concentration tested when compared to control values, either in the presence or absence of S9 metabolic activation.

Positive controls gave the expected increases in the frequency of mutations, indicating the efficacy of the metabolic activation mix and the sensitivity of the test procedure.

Chromosome aberration test in cultured Chinese hamster cells (Izumi, 1996)

Test article

The test article, Lipase AY, lot no. LAY-N48-002 was used. The activity was 142000 U/g. Lipase AY is produced by *C. rugosa*.

Study design

The potential of lipase AY 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, both in absence and presence of S9, the cells were treated for six hours and the harvest time was 18 hours after treatment stopped. In an additional dose finding study, in the absence of S9, the cells were treated for 24 or 48 hr. Since in toxicity experiments effects on the mitotic index were absent, the treatment levels in the main studies were 1250, 2500 and 5000 μg/ml in the absence or presence of metabolic activation for six hours. As negative results were obtained, a second experiment in the absence of S9 was performed using a continuous treatment until harvest at 24 or 48 hours.

Test	*Test material*	*Concentration*	*Test object*	*Result*
chromosome aberration (*In vitro*)	Lipase AY	0, 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

A516 – LIPASE FROM *CANDIDA RUGOSA* AS A PROCESSING AID (ENZYME)

Introduction

FSANZ received an application from Biocatalysts Ltd to amend the Code to approve a new source, the yeast *C. rugosa*, 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 *C. rugosa*.

It 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. That enzyme is sourced from bovine stomach; salivary glands or forestomach of calf, kid or lamb; porcine or bovine pancreas.

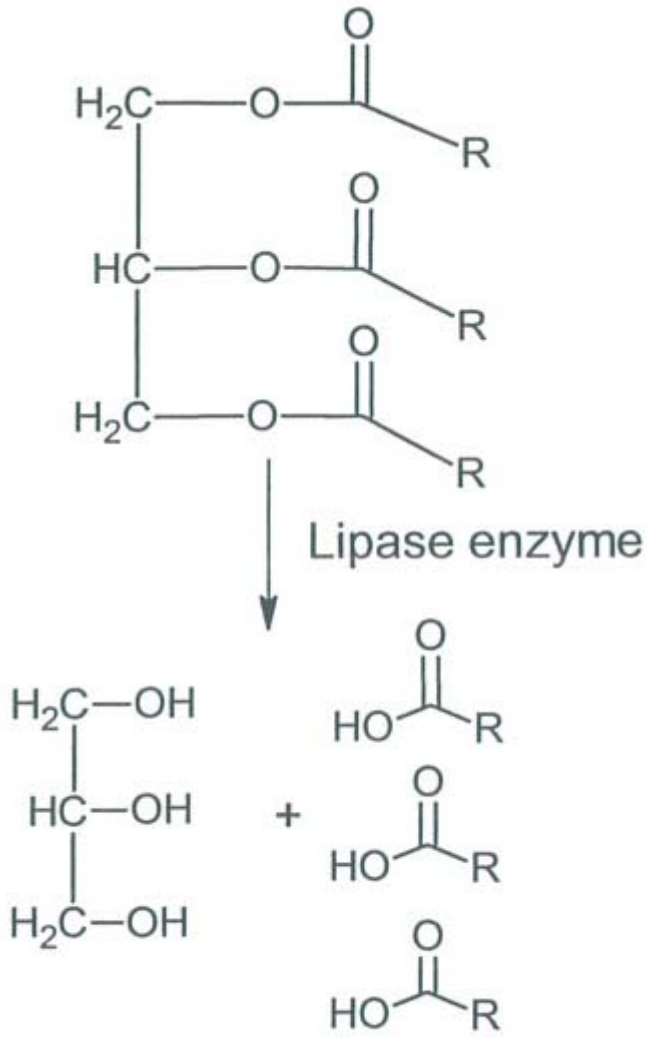
The enzyme for this Application is obviously from a microbial source (the yeast *C. rugosa*) rather than an animal source.

The enzyme preparation is a white powder with pH stability between 3 and 8 and optimum pH between 6-7. The optimum temperature of use is between 40 and 50ºC. It is thermally stable below 37ºC in an aqueous solution. The molecular weight of the enzyme is 60,000 Daltons determined by SDS PAGE.

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 all 3 triglyceride positions so it is able to cleave three fatty acids (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 consumers with preferences for vegetarian and kosher foods.

The Applicant claims lipase sourced from *C. rugosa* is a broad spectrum lipase which attacks all three triglyceride positions, in both hard and soft fats. However it has more specific activity for short chain fatty acids, in particular C4 (butyric acid). Its specific use and justification for use is to produce cheese flavours.

Typical cheese characteristic flavours include short chain fatty acids of the C4 and C5 length such as butyric acid and isovaleric acid which because they are short chain lengths are more volatile and produce sharp/tangy 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. 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 *Candida rugosa*. 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 *C. rugosa* 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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Attachment 5

Microbiological assessment report

Candida nomenclature

Task

The Applicant for Application A516 – Lipase from *C. cylindracea* as a processing aid (enzyme) has called the new microbial yeast enzyme source *C. cylindracea*.

To ensure the newer nomenclature is correct and appropriate the common nomenclature for this organism has been reviewed.

Research Results

The research involved a literature search, internet scans and direct enquiries to experts. The results are summarised below:

A personal communication from Associate Professor Warren Shipton of James Cook University indicated that *C. cylindracea* was listed in Barnett et al. (2000) with no synonyms and that *C. rugosa* was listed with only changes in its genus state.

A search of the National Collection of Yeast Cultures collection (http://www.ncyc.co.uk/action.lasso) revealed that there is no listing for *C. cylindracea.* However, there are six listings for *C. rugosa.*

The ExPASy (Expert Protein Analysis System) proteomics server of the Swiss Institute of Bioinformatics (SIB) (http://www.expasy.org) contains listings for *C. rugosa* with *C. cylindracea* listed in brackets.

UniProt, the universal protein knowledgebase, (http://www.ebi.uniprot.org) contains reference to proteins derived from *C. rugosa*, with *C. cylindracea* in brackets.

The Deutsche Sammlung von Microorganismen und Zellbulturen GmbH (http://www.dsmz.de.species.sp300215.htm) lists both *C. rugosa* and *C. cylindracea.* Both references cross-reference to each other.

The American Type Culture Collection (http://www.atcc.org) lists 8 entries when searched for *C. rugosa*, of which one is listed as *C. cylindracea.*

The Stevens Laboratory within the Scripps Research Institute Department of Molecular Biology and Chemistry (http://stevens.scripps.edu/ESGDB/EC_3.html) under the information for search information for lipases, contains reference to the lipases being sourced from '...yeast (*Candida rugosa*) (formerly *Candida cylindracea*)...'.

The Centraalbureau voor Schimmelcultures, an Institute of the Royal Netherlands Academy of Arts and Sciences (http://www.cbs.knaw.nl) lists *C. rugosa* with the taxonomic information stating '…currently recognised species…'. For *C. cylindracea*, it indicates name changes of *C. rugosa* and *C. zeylanoides*.

A recent publication by Ferrer (2001, pp 223) states '…*C. rugosa* (formerly *C. cylindracea*)…', which supports the suggestion of a name change for this species.

A personal communication from Dr Ailsa Hocking, Section Leader, Mycology & Mycotoxins, Food Science Australia indicates that these two yeast are regarded as two separate species, though they both produce lipases. *C. cylindracea* was considered a synonym of *C. zeylanoides* but was restored as a separate species in 1998 because of a significant difference in the mole percentage guanine + cytosine of DNA (mol% GC). The references quoted are Barnett et al. (2000) and Boekhout et al. (2002).

Further personal communication with Dr Hocking indicated that she could not support a decision to use *C. rugosa* instead of *C. cylindracea* as in her opinion they are both valid species. Dr Hocking provided a further reference for this opinion in Kurtzman and Fell (1998).

Conclusion

It is apparent that the situation with the nomenclature of this species of yeast is unclear. Advice from Dr Ailsa Hocking, a renowned Australian mycology expert, indicates that it would be unwise to use the term *C. rugosa* in preference to *C. cylindracea*. It is Dr Hocking's expert opinion that both are valid species. Dr Hocking supports her decision with references from Barnett et al. (2000), Boekhout et al. (2002) and Kurtzman and Fell (1998).

The various world cell databanks that were searched were not conclusive in the nomenclature of these two yeast species. Whilst the Centraalbureau voor Schimmelcultures, a trusted institute in terms of yeast taxonomy, indicates that *C. cylindracea* has undergone a name change of *C. rugosa*, this position is at odds with the respected references utilised by Dr Hocking.

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