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

7-06
4 October 2006

DRAFT ASSESSMENT REPORT

PROPOSAL P276

REVIEW OF PROCESSING AIDS (ENZYMES)

DEADLINE FOR PUBLIC SUBMISSIONS: 6pm (Canberra time) 29 November 2006
SUBMISSIONS RECEIVED AFTER THIS DEADLINE
WILL NOT BE CONSIDERED
(See 'Invitation for Public Submissions' for details)

For Information on matters relating to this Assessment Report or the assessment process generally, please refer to http://www.foodstandards.gov.au/standardsdevelopment/

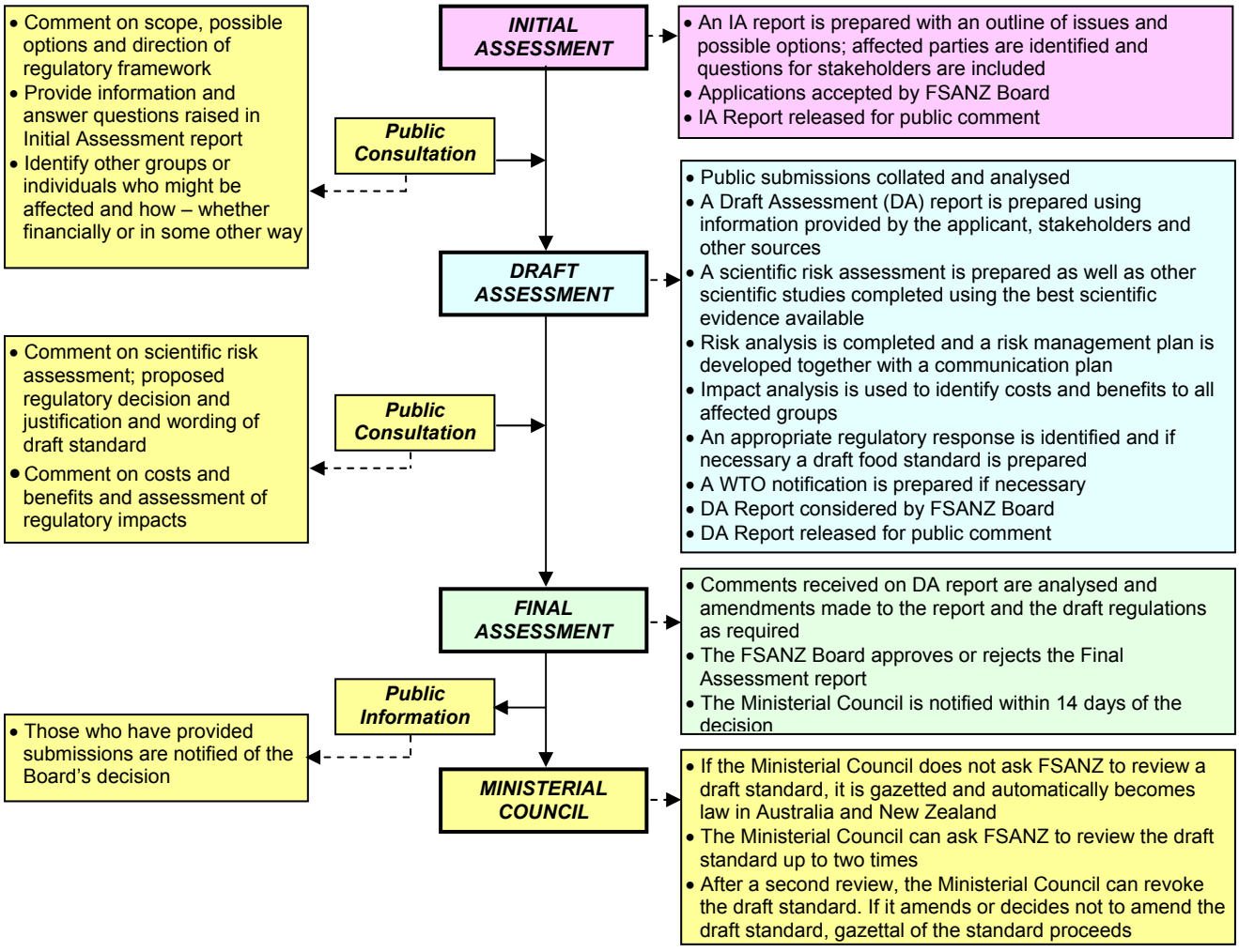
FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

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 Proposal P276; and prepared a draft variation to the *Australia New Zealand Food Standards Code* (the Code).

FSANZ invites public comment on this Draft Assessment Report based on regulation impact principles and the draft variation to the Code for the purpose of preparing an amendment to the Code for approval by the FSANZ Board.

Written submissions are invited from interested individuals and organisations to assist FSANZ in preparing the Final Assessment for this Proposal. 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 should be received by FSANZ by 6pm (Canberra time) 29 November 2006.

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

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

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

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Executive Summary and Statement of Reasons

FSANZ has initiated Proposal P276 – Review of Processing Aids (Enzymes) to review clauses 15, 16 and 17 of Standard 1.3.3 – Processing Aids of the Code. A separate Proposal, P277 – Review of Processing Aids, is currently reviewing in parallel the regulation of processing aids other than enzymes. Proposal P276 focused on the review of enzymes separately from the other processing aids since FSANZ considers the safety assessment, risk management and technical issues are different for enzymes compared to other processing aids.

Proposal 276 will not lead to a restructure of Standard 1.3.3 (clauses 15, 16 and 17). The basic structure of the Standard was developed during the course of two earlier proposals and is considered appropriate.

Safety of currently permitted enzyme processing aids

A total of seven enzyme-processing aids have been evaluated for their safety. These enzymes had been recently reviewed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). All substances were determined to have low oral toxicity and were considered to raise no safety concerns. A list of these enzymes appears below.

Substance
Alpha-acetolactate decarboxylase from *Bacillus brevis* expressed in *Bacillus subtilis*
Alpha-amylase from *Bacillus licheniformis*
Hexose oxidase from *Chondrus crispus* expressed in *Hansenula polymorpha*
Invertase from *Saccharomyces cerevisiae*
Maltogenic amylase from *Bacillus stearothermophilus* expressed in *Bacillus subtilis*
Xylanase from B*acillus subtilis*
Mixed xylanase, *beta*-glucanase enzyme preparation, produced by a strain of *Humicola insolens*

Removing obsolete enzymes

At Initial Assessment, some submitters suggested caution against deleting the use of any enzymes not currently used in Australia or New Zealand to ensure any deletions would not preclude product being imported into Australia and New Zealand. FSANZ has been unable to specifically identify enzymes that may be candidates for deletion and proposes the following:

FSANZ requests that industry identify if there are any obsolete processing aids which are no longer used, or likely to ever be used again in the food industry, in either Australia or New Zealand. Possible use internationally must also be considered since our trading partners export food products to both Australia and New Zealand and these products must also meet the requirements of the Code. If none are identified, then FSANZ will retain the current permissions.

Nomenclature

The Initial Assessment for this Proposal asked interested parties to identify any errors, clarify nomenclature, remove duplications and anomalies, improve consistency between this standard and other parts of the Code and improve the general operation and function of the Standard.

FSANZ has undertaken a review of the nomenclature of currently permitted enzymes as processing aids, compared to international approvals and bodies charged with reviewing nomenclature of enzymes, e.g., the International Union of Biochemistry and Molecular Biology (IUBMB). Proposed amendments to correct and simplify the names of specific enzymes have been made.

Issues raised in submissions

Following the public comment period (17 December 2003 till 25 February 2004) ten submissions were received. Nine supported the intent of the review, while one raised issues beyond the mandate of the review. Comments were received on the safety of currently approved enzymes in the Code. In addition, some stakeholders suggested that a broader, more generic approval system be adopted so as to include enzymes that had been evaluated and approved by other international agencies as currently approved enzymes in the Code. This Proposal is not a vehicle to give approvals for new, currently non-approved enzymes. Applicants would still need to make applications to request permissions to use new enzymes in the Code. However, FSANZ is satisfied that all currently permitted enzymes are safe for use. No submissions were received addressing the overall structure of the standard.

FSANZ has sought and still seeks further comments from interested stakeholders on the following, but not exclusively limited, to:

- new scientific evidence regarding the safety of particular enzymes;
- recent international regulatory changes which may impact on specific enzyme processing aids;
- enzymes which are no longer used or likely to be used in the future;
- names of approved enzymes to better reflect current usage and international standards;
- errors and anomalies within the Standard; and
- specific amendments proposed in this report, in particular:
  updated enzymes (and their microbial source) being used in place of bromelain EC 3.4.22.4, carboxyl proteinase EC 3.4.23.6, metalloproteinase EC 3.4.24.4, and serine proteinase EC 3.4.21.14.

External Advisory Group

An External Advisory Group (EAG) was established to assist FSANZ with this review. Members were drawn from experts from industry groups, regulatory agencies, academic and consumer groups with expertise in food enzymes and their regulation. FSANZ staff met with the EAG in May 2004. Expert advice was received on the proposed amendments with further information received after this meeting via email correspondence.

Preferred Approach

FSANZ has reviewed clauses 15, 16 and 17 of Standard 1.3.3 – Processing Aids and has proposed a number of draft variations. These are proposed to ensure public health and safety, correct errors, remove duplications and anomalies, ensure consistency and improve the function of the Standard.

Statement of Reasons

The draft variations to Standard 1.3.3 – Processing Aids of the Code are recommended for the following reasons:

- The proposed amendments ensure the protection of public health and safety.

- The proposed amendments ensure consistency within the Code and improved consistency, as far as is possible, with other international food standards.

- The proposed amendments have included information and submissions on issues received, as well as advice from an Expert Advisory Group, made up of experts external to FSANZ.

- There will not be any expected added costs to food manufacturers, consumers or regulatory agencies arising from these proposed amendments.

- There are no other alternatives that are more cost effective than the proposed amendments to the Code.

FSANZ therefore seeks comments on this Draft Assessment Report, which will assist it in preparing the Final Assessment.

1. Introduction

Standard 1.3.3 – Processing Aids of the *Australia New Zealand Food Standards Code* (the Code) was developed during the review of the former Australian *Food Standards Code* and the *New Zealand Food Regulations 1984.* The Standard is a joint Australia and New Zealand Standard and is based on Standard A16 from the former Australian *Food Standards Code.*

Standard A16 was taken up in Standard 1.3.3 without a comprehensive review as it had relatively recently been developed and gazetted in 1996. This Proposal, P276, is to review clauses 15, 16 and 17 of Standard 1.3.3 to review permissions for enzymes as processing aids and to formally harmonise the Australian and New Zealand regulations under the Code.

A separate Proposal, P277 – Review of Processing Aids (other than Enzymes), is running concurrently to comprehensively evaluate the full list of processing aids permissions not formally done when the review of food standards of both Australia and New Zealand was undertaken.

Proposal P276 focused on the review of enzymes, separately from the other processing aids since FSANZ considers the safety assessment, risk management and technological issues are different from enzymes as distinct from other processing aids.

2. Regulatory Problem

2.1 Current Standard

The regulation of processing aids for all food in the Code is covered by Standard 1.3.3 – Processing Aids. This Standard regulates the use of processing aids in food manufacture, prohibiting their use in food unless there is a specific permission within this Standard. Processing aids are defined in this Standard in clause 1 as:

> **processing aid** means a substance listed in clauses 3 to 18, where –
>
> (a) the substance is used in the processing of raw materials, foods or ingredients, to fulfil a technological purpose relating to treatment or processing, but does not perform a technological function in the final food; and
>
> (b) the substance is used in the course of manufacture of a food at the lowest level necessary to achieve a function in the processing of that food, irrespective of any maximum permitted level specified.

The three different categories of enzymes within Standard 1.3.3 are listed in:

- clause 15, enzymes of animal origin;
- clause 16, enzymes of plant origin; and
- clause 17, enzymes of microbial origin.

The enzymes are permitted for food manufacturing needs, provided that the enzyme is derived from the corresponding source or sources specified in the Table. For enzymes derived from microbial source, the microbial source organism may contain additional copies of genes from the same organism.

2.2 Matters for review

Matters being considered as part of this review include:

- safety of currently approved enzymes and by-products of enzymatic reactions and the guidelines for the safety assessment of enzymes;
- nomenclature used for enzymes and source organisms;
- enzymes not currently used in Australia and New Zealand; and
- other issues raised by submitters following the first round of public consultation

However, this review will not be used as a mechanism for the approval of new enzymes in Australia and New Zealand. New enzymes will need to go through the normal application process.

3. Objective

The objective of this proposal is to ensure that Standard 1.3.3 provides appropriate permissions for enzymes used in Australia and New Zealand.

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.

The main section 10 objectives that this Proposal will meet are to ensure the protection of public health and safety and that any amendments to the Standard are based on the best available scientific evidence. Other objectives are to ensure consistency, as far as possible, between domestic and international regulations of enzymes. This objective aims to ensure an efficient and internationally competitive food industry.

4. Background

4.1 Historical Background

The former National Food Authority (NFA) proposed (Proposal P86 – Development of a Standard to regulate the Use of Processing Aids) the development of a standard for processing aids for Australia in 1995 (Standard A16).

Proposal P86 was considered by the NFA in 1995 and Standard A16 was gazetted in the former Australian *Food Standards Code* in April 1996.

Prior to Standard A16 being included in the Code, processing aids were regulated in a mixed fashion. Some were listed in the *NHMRC Supplement to the Code* and others were individually approved and incorporated in the specific commodity standard.

A subsequent Proposal by the former Australia New Zealand Food Authority (ANZFA), P188 – Processing Aids, as part of its review of the *Australian Food Standards Code,* developed Standard 1.3.3 – Processing Aids of the Code, which included enzyme permissions. The Preliminary Assessment Report for P188 was released for public comment in October 1998, while the Full Assessment Report was released in August 1999. The Inquiry Report was released in December 1999 and the subsequent standard, Standard 1.3.3, was gazetted on 20 December 2000 (as part of the Code).

The Inquiry Report (now termed the Final Assessment Report) for Proposal P188 stated that in relation to enzymes:

- The sources listed in the Table to clause 17 (enzymes from microbial origin) may contain additional copies of genes from the same organism.

- Enzymes from microbiological sources are not permitted to be derived from combinations of the approved sources for that particular enzyme without a specific listing.

- Any additional permission will require separate formal applications to ANZFA (now FSANZ).

4.2 Regulation of enzymes internationally

The regulation of enzymes for Australia and New Zealand are contained within Standard 1.3.3 – Processing Aids of the Code, specifically, clauses 15, 16 and 17 of Standard 1.3.3 as described in section 2.1 above. That is, enzymes are considered processing aids.

However, the regulation of enzymes internationally is quite varied between countries, with specific countries either requiring a full approval process, a notification of the enzyme or no approval/notification requirements. Pre-market approval may depend on whether an enzyme is classified as a processing aid or a food additive, although the common element regardless of classification is that the safety of the enzyme must be assured.

In summary, international regulation of processing aids (which includes enzymes) is as follows:

4.2.1 Codex

The Codex definition considers that processing aids are regarded as a subset of food additives; however, Codex does not have a specific standard for processing aids but has an inventory of processing aids. Genetically modified organisms derived enzyme preparations are assessed for safety using the same scheme as non-GMO derived organisms.

4.2.2 Canada

Enzymes are regulated as food additives by Health Canada according to the Food and Drug Acts and require pre-approval before food can be marketed containing enzymes. Specific enzymes are listed by source, allowed applications and limits of use.

4.2.3 Japan

The Ministry of Health, Labor and Welfare regulate enzymes as food additives. Enzymes that are not listed on the food additives list require a pre-market approval.

4.2.4 USA

In the USA the Code of Federal Regulations separately regulates the use of food additives and processing aids. Enzyme preparations are regulated either as 'secondary' direct food additives under Title 21 of the Code of Federal Regulations (CFR), Part 173, or are affirmed as GRAS (Generally Recognised As Safe) substances in 21 CFR Part 184. The regulatory status of food additives or substances affirmed as Generally Recognised as safe (GRAS) is established through the petition process.

4.2.5 EU

In the EU regulatory framework, a distinction is made between food additives (essentially substances which are added to food and have a technological function in that food) and processing aids (essentially substances which are added during food processing and may end up in the food but do not have a technological function in the processed foodstuff). With a few exceptions, food enzymes are considered to be processing aids and not food additives.

The regulations on food additives have been harmonised by a number of EC Directives adopted in 1994. However, there is as yet no harmonised EU legislation on processing aids in general or food enzymes in particular. This means that the application of enzymes in food is governed by legislation at a national level, which differs widely.

Some examples:

- Denmark and France have a separate legislation on enzymes, requiring an implicit or explicit authorisation (enforcing SCF guidelines).

- Germany considers enzymes to be food additives but exempts them from approval (authorisation).

- The Netherlands does not have a specific enzyme regulation, but enzymes from genetically modified micro-organisms are covered by the Dutch regulation on Novel foods, requiring an authorisation. (*Note: approvals for genetically modified enzymes have not been authorised until July 2003).*

- In the United Kingdom, there are no specific regulations relating to the use of enzymes as processing aids, but a voluntary system is in place to evaluate the safety of new enzyme preparations.

Moreover, all Member States have standards for specific foods e.g. for bread and other bakery products, cheese, beer, etc. In this so-called vertical legislation, often provisions for the use of enzymes are included.

5. Relevant Issues

5.1 Safety of currently permitted enzymes

Submissions to P276 raised the following issues in regard to safety of enzymes:

- Enzymes which have been evaluated and approved by other international agencies according to international standards such as the former European Scientific Committee for Food (SCF) guidelines or equivalent, should be exempted from the planned safety review by FSANZ.

- Food enzymes have been shown from history to be inherently safe and there is no need to evaluate enzymes not examined since 1996, except those that have been identified by FSANZ or other appropriate parties to have a toxicological concern.

- Enzymes that interact with a wide range of substrates may pose safety concerns due to production of unwanted or undesirable chemical products.

5.1.1 Evaluation

The National Food Authority (NFA) created the early processing aids standard (A16 in the former *Australia Food Standards Code*) via proposal P86 in 1996, the evaluations for enzymes for this proposal have focused on post 1996 enzyme evaluations.

The following criteria have been used to determine which enzymes were evaluated for this Proposal.

(i) the enzyme has been (re)-evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), or another scientific agency[1] since 1995; or

(ii) the substance has been identified by FSANZ, or other parties, as of potential toxicological concern.

Using these criteria, a total of seven enzyme processing aids have been evaluated for their safety. The substances that were selected had been relatively recently evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Each of the selected substances was reviewed, using evaluation reports from other (inter)-national organisations or agencies, where these were available. All were determined to have low oral toxicity and were considered to raise no safety concerns. A list of the enzymes assessed is provided in Table 1.

[1] e.g. National Industrial Chemicals Notification and Assessment Scheme (NICNAS), National Health and Medical Research Council (NHMRC), European Scientific Committee on Food (SCF), European Food Safety Authority (EFSA), the United Stated Environmental Protection Agency (US EPA), Agency for Toxic Substances and Disease Registry (ATSDR), International Agency for Research on Cancer (IARC), and the Environmental Health Criteria (EHC)

Table 1: Enzymes assessed to have no toxicological concerns

Substance
Alpha-acetolactate decarboxylase from *Bacillus brevis* expressed in *Bacillus subtilis*
Alpha-amylase from *Bacillus licheniformis*
Hexose oxidase from *Chondrus crispus* expressed in *Hansenula polymorpha*
Invertase from *Saccharomyces cerevisiae*
Maltogenic amylase from *Bacillus stearothermophilus* expressed in *Bacillus subtilis*
Xylanase from B*acillus subtilis*
Mixed xylanase, *beta*-glucanase enzyme preparation, produced by a strain of *Humicola insolens*

On the issue of approval of enzymes and the creation of undesirable by-products due to the wide range of substrates that some enzymes may interact with, FSANZ's view is that this has to be assessed on a case-by-case basis as enzymes are usually approved for use on a broad range of substrates in the Code. This situation may apply when the substrate specificity of the enzyme is low and it would be necessary to assess the toxicity of by-products derived from a broader range of use in foods.

A detailed safety assessment report is as per **Attachment 2**

5.2 Removing any obsolete enzymes

Some submitters suggested caution against deleting the use of any enzymes not currently used in Australia or New Zealand to ensure any deletions would not inhibit international trade.

5.2.1 Evaluation

FSANZ requested information from submitters on this point in the Initial Assessment Report. One submitter made the comment that they did not support the removal of processing aids that may be considered obsolete, since it is impossible to determine when a processing aid may be required for use in the future, and to predict for what purposes the enzyme is required. Also removing 'obsolete' processing aids for Australia and New Zealand industries from the Code may cause trade issues if other countries that still use that processing aid in food exported to either country. Another submitter cautioned that enzymes should only be obsolete if they are not currently used in any country (not just Australia or New Zealand) to not inhibit international trade.

FSANZ invites industry to identify if there are any obsolete enzymes which are no longer used, or likely to ever be used again in the food industry, in either Australia or New Zealand. Possible use internationally must also be considered since our trading partners export food products to both Australia and New Zealand and these products must also meet the requirements of the Code. If none are identified, then FSANZ will retain the current permissions.

5.3 Correct errors, remove anomalies and improve nomenclature of enzymes

The structure of Standard 1.3.3 will not be changed by this proposal. The structure of the standard was resolved during the earlier two Proposals, P86, that developed A16 in the former Australian *Food Standards Code* and P188, which developed the current Standard 1.3.3 in the Code.

Both these Proposals were undertaken involving consultation with various interested parties from the food industries and regulatory agencies. There were full rounds of public consultations and submissions to both proposals.

The Initial Assessment for this proposal asked interested parties to make suggestions to correct any errors, clarify nomenclature, remove duplications and anomalies, improve consistency between this standard and the rest of the Code and improve the general operation and function of the standard. No submissions were received addressing the overall structure of the Standard.

5.3.1 Naming and classifying enzymes

FSANZ has undertaken a review of the nomenclature of currently permitted enzymes as processing aids, referring to the recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB). The proposed amendments are summarised in Tables 2-4 below and detailed consideration of the nomenclature appears in **Attachment 3**.

Table 2: Permitted enzymes of animal origin

Suggested action	Reason, comment
Amend the entry for Lipase to read 'Lipase, triacylglycerol'.	This entry was considered together with all the other 'lipase' entries. This amendment gives a consistent approach to listing lipase in the Code, while providing a more accurate description of its function as a lipase.
Amend the entry for Pepsin by reposition the letters 'EC' so that they appear on a new line immediately before the actual enzyme numbers.	This corrects a minor editorial error.

Table 3: Permitted enzymes of plant origin

Suggested action	Reason, comment
Amend the entry for Bromelain by replacing it with 'Bromelain, stem EC 3.4.22.32'.	The IUBMB deleted the entry for bromelain (EC 3.4.22.4) in 1992 and transferred it to two separate entries: stem bromelain (EC 3.4.22.32) and fruit bromelain (EC 3.4.22.33). Since the original entry in the Code was for bromelain sourced from pineapple stem (*Ananas comosus*) it was agreed to limit the entry to stem bromelain. It was noted that extending the permission to fruit bromelain was beyond the scope of the review.

Suggested action	Reason, comment
Delete the full entry for 'malt carbohydrases…' and include individual entries for α-amylase and ß-amylase derived from malted cereals. This will result in a new entry for α-amylase from malted cereals and an additional plant source entry - 'malted cereals' for ß-amylase.	We supported the rationale put forward by Enzymes Solutions that, as it was permissible to use the two enzymes together, even though they are sourced separately, then it was sensible to provide for separate permissions.
Insert 'EC 3.4.22.14' in association with the entry for Actinidin.	This corrects the omission of an EC number for actinidin.

Table 4: Permitted enzymes of microbial origin

Suggested action	Reason, comment
Amend the entry for 'aminopeptidase' to read 'leucyl aminopeptidase.	The current nomenclature is not consistent with IUBMB. Aminopeptidase is the name of all enzymes in the 3.4.11.x reaction category of which there are 20 different amino peptidases listed. The enzyme, EC 3.4.11.1 is commonly referred to as 'leucyl aminopeptidase'. Comment will be sought from industry to confirm that leucyl aminopeptidase (from the source enzymes listed) is the actual enzyme being used and not a different aminopeptidase.
Amend the entry of α-amylase so that (a) all occurrences of '*Bacillus stearothermophilus*' will now read '*Geobacillus stearothermophilus*'; and (b) the entry *Bacillus subtilis* is replaced with *Bacillus amyloliquefaciens.*	Amendment (a) brings the name of the microbial source up-to-date with current bacterial nomenclature. Amendment (b) recognises *Bacillus amyloliquefaciens* as a separate species from *Bacillus subtilis* and therefore the entry for *B. subtilis* should be replaced with *Bacillus amyloliquefaciens.*
Amend the entry for β-amylase so that the entry *Bacillus subtilis* is replaced with *Bacillus amyloliquefaciens.*	This amendment recognises that *Bacillus amyloliquefaciens* as a separate species from *Bacillus subtilis* and therefore the entry for *B. subtilis* should be replaced with *Bacillus amyloliquefaciens.*
Amend the entry for Arabinase to read 'Endo-arabanase'.	The entry 'endo-arabanase' is a more specific name for this enzyme.
Amend the entry for Arabino-furanosidase to read 'α-Arabinofuranosidase'.	This is a minor change that more accurately reflects the specificity of the enzyme.

Suggested action	Reason, comment
Delete the entry for Carboxyl proteinase EC 3.4.23.6 and replace it with: (a) aspergillopepsin I (EC 3.4.23.18) derived from *Aspergillus niger* and *Aspergillus oryzae;* (b) aspergillopepsin II (EC 3.4.23.19) derived from *Aspergillus niger;* and (c) mucorpepsin (EC 3.4.23.23) derived from *Rhizomucor miehei.*	IUBMB entry for carboxyl proteinase EC 3.4.23.6 was transferred to 12 different enzymes and was subsequently deleted in 1992. It is proposed to update the entry for carboxyl proteinase based on industry advice about which of the 12 more specifically defined enzymes that replace carboxyl proteinase, are being used by industry. Acceptance of this advice will also be dependent on industry being able to provide official documentation confirming the specific microbial source(s) of the replacement enzymes. Industry members of the External Advisory Group advised that: aspergillopepsin I (EC 3.4.23.18) and aspergillopepsin II (EC 3.4.23.19) derived from *Aspergillus niger* and/or *Aspergillus oryzae;* and mucorpepsin (EC 3.4.23.23) derived from *Rhizomucor miehei* were suggested replacements for carboxyl proteinase.
Replace the entry for 'Esterase' with 'Carboxylesterase'	The entry 'carboxylesterase' is a more specific name for this enzyme and is the listed IUBMB common name.
Replace the entry for 'Glucose isomerase or glucose isomerase xylose isomerase' with 'xylose isomerase'	This simplifies the entry while maintaining consistency with the IUBMB enzyme nomenclature.
Amend the entry α-Glucosidase (maltase), by deleting '(maltase)'.	This simplifies the entry while remaining consistent with IUBMB Enzyme nomenclature.
Replace 'β-Glucosidase exo-1,3' with 'glucan 1,3-β-glucosidase'.	This aligns with IUBMB Enzyme nomenclature, while not changing the level of complexity of the entry.
Amend the entry for 'Hemicellulase endo-1,3-β-xylanase or xylanase' by deleting the words 'Hemicellulase' to leave the entry 'Endo-1,3-β-xylanase'.	This removes the outdated term 'hemicellulase' while remaining consistent with IUBMB Enzyme nomenclature.
Amend the entry for 'Hemicellulase Endo-1,4- β-xylanase or xylanase' by (a) deleting the words 'Hemicellulase' & 'or xylanase' to leave the entry: **'**Endo-1,4-β-xylanase'; (b) replacing the entry *Bacillus subtilis* with *Bacillus amyloliquefaciens*; and (c) in the source column replace 'α' with 'β'.	These amendments will: (a) simplify the entry by deleting the outdated term 'hemicellulase' while remaining consistent with IUBMB Enzyme nomenclature; (b) recognising that *Bacillus amyloliquefaciens* as a separate species from *Bacillus subtilis* and therefore the entry for *B. subtilis* is replaced with *Bacillus amyloliquefaciens*; and (c) correct an editorial error.

Suggested action	Reason, comment
Amend the entry for 'Hemicellulase multicomponent enzyme' by (a) replacing the words 'Hemicellulase multicomponent enzyme' with 'Endo-1,4-β-mannanase'; and (b)) replacing the entry *Bacillus subtilis* with *Bacillus amyloliquefaciens.*	These amendments (a) removes the broad and outdated term 'hemicellulase multicomponent enzyme' and replaces it with a more accurate and up-to-date entry with respect to the IUBMB enzyme nomenclature; and (b) recognises *Bacillus amyloliquefaciens* as a separate species from *Bacillus subtilis* and therefore the entry for *B. subtilis* should be replaced with *Bacillus amyloliquefaciens.*
Amend the entry 'Lactase β-Galactosidase' by deleting the word 'lactase'.	This simplifies the entry while remaining consistent with IUBMB Enzyme nomenclature.
Amend the entry for maltogenic amylase by (a) inserting 'α-' so that it reads 'Maltogenic α-amylase'; and (b) replacing *Bacillus stearothermophilus* with *Geobacillus stearothermophilus*.	(a)This is a more accurate description of the enzyme while maintaining consistency with the IUBMB enzyme nomenclature. (b) This updates the bacterial nomenclature for the source organisms.
Delete the entry for metalloproteinase (EC 3.4.24.4) and replace it with: Bacillolysin (EC 3.4.24.28) produced from *Bacillus amyloliquefaciens;* Bacillolysin (EC 3.4.24.28) produced from *Bacillus subtilis*; Thermolysin (EC 3.4.24.27) produced from *Aspergillus oryzae*; and Deuterolysin (EC 3.4.24.39) produced from *Aspergillus oryzae.*	IUBMB entry for metalloproteinase (EC 3.4.24.4) was transferred to 10 different enzymes and the entry was subsequently deleted in 1992. It is proposed to update the entry for metalloproteinase based on industry advice about which of the 10 more specifically defined enzymes that replace metalloproteinase, are being used by industry. Acceptance of this advice will be dependent on industry being able to provide official documentation confirming the specific microbial source(s) of the replacement enzymes. Industry members of the External Advisory Group advised that metalloproteinase should be replaced with: Bacillolysin (EC 3.4.24.28) produced from *Bacillus amyloliquefaciens;* Bacillolysin (EC 3.4.24.28) produced from *Bacillus subtilis*; Thermolysin (EC 3.4.24.27) produced from *Aspergillus oryzae*; and Deuterolysin (EC 3.4.24.39) produced from *Aspergillus oryzae*
Amend the entry for pectin lyase to read 'Pectin lyase EC 4.2.2.10'.	Minor editorial change.
Amend the entry for 'Pectin methylesterase or Pectinesterase' by deleting 'Pectin methylesterase' to retain the word 'Pectinesterase'.	This simplifies the entry while remaining consistent with IUBMB enzyme nomenclature.

Suggested action	Reason, comment
Amend the entry for '6-phytase' by replacing the digit '6' with '4' for both occurrences of '6-phytase'.	This simplifies the entry while remaining consistent with IUBMB Enzyme nomenclature. Specifically seek comments from industry on the implication of this amendment.
Amend the entry for 'Polygalacturonase or Pectinase multicomponent enzyme' by deleting the words 'or Pectinase multicomponent enzyme'.	This simplifies the entry while remaining consistent with IUBMB Enzyme nomenclature.
Delete the entry for serine proteinase and insert an entry for Subtilisin EC 3.4.21.62 sourced from *Bacillus halodurans*** or *Bacillus licheniformis* ** Refer to full discussion at attachment 3.	IUBMB entry for serine proteinase (EC 3.4.21.14) was transferred to 5 different enzymes and was subsequently deleted in 1992. It is proposed to update the entry for serine proteinase based on industry advice about which of the 5 more specifically defined enzymes that replace serine proteinase, are being used by industry. Acceptance of this advice will be dependent on industry being able to provide official documentation confirming the specific microbial source(s) of the replacement enzymes. Industry members of the External Advisory Group advised that serine proteinase should be replaced with: Subtilisin (EC 3.4.21.62) derived from either *Bacillus halodurans*** or *Bacillus licheniformis*
Transglucosidase EC 2.4.1.24 No change.	Seek comment from industry on whether the simplicity of the current entry is sufficiently accurate given that it is not consistent with the IUBMB nomenclature.
Amend the Editorial Note by (a) deleting the sentence: '*Bacillus subtilis* covers the strain known under the name *Bacillus amyloliquefaciens*.'; and (b) inserting the sentence: '*Bacillus stearothermophilus* is the former name for *Geobacillus stearothermophilus.'*	(a) Since 1987, *B. amyloliquefaciens* is described as a separate species and therefore should be treated as such in the table to clause 17. This sentence has become obsolete and can now be deleted. Stakeholder comment is supported that the entry for *Bacillus stearothermophilus* be updated to reflect its current name: *Geobacillus stearothermophilus.*

5.3.2 Enzyme Commission Numbers

All enzymes listed in the Code make reference to an enzyme commission number. The Nomenclature Committee advise that when citing an enzyme number it should be preceded by EC and a space. As this should be done without the use of the square brackets, all the enzyme commission numbers have been amended to reflect this.

5.3.3 Obsolete enzyme nomenclature

The chemical reaction catalysed by an enzyme is the specific property that distinguishes one enzyme from another and this is the basis for the classification and naming of enzymes used by the IUBMB. The IUBMB also allocate a four digit EC number on this basis. The first three digits define the reaction catalysed and the fourth digit provides a unique serial number.

In 1992, the IUBMB published revised recommendations of the nomenclature committee of the IUBMB. This edition included a comprehensive review of the subclass 3.4, the peptidases. This has resulted in a number of enzymes entries being reclassified and transferred to other entries. These changes effect four enzyme permissions listed in the Code, namely:

bromelain EC 3.4.22.4,
carboxyl proteinase EC 3.4.23.6,
metalloproteinase EC 3.4.24.4, and
serine proteinase EC 3.4.21.14.

The classification of these enzymes has been transferred to a variety of more specific and recently classified enzymes listed in table 5 below.

Table 5: IUBMB Enzyme nomenclature committee reclassified of four specific enzymes found in the Code

Current enzyme entry in the Code	**Updated enzyme reclassification and names based on IUBMB review of peptidases**
bromelain EC 3.4.22.4	EC 3.4.22.32 stem bromelain EC 3.4.22.33 fruit bromelain
carboxyl proteinase EC 3.4.23.6	EC 3.4.23.18 aspergillopepsin I; EC 3.4.23.19 aspergillopepsin II; EC 3.4.23.20 penicillopepsin; EC 3.4.23.21 rhizopuspepsin EC 3.4.23.22 endothiapepsin EC 3.4.23.23 mucorpepsin EC 3.4.23.24 candidapepsin EC 3.4.23.25 saccharopepsin EC 3.4.23.26 rhodotorulapepsin EC 3.4.23.27 physaropepsin EC 3.4.23.28 acrocylindropepsin EC 3.4.23.30 pycnoporopepsin
metalloproteinase EC 3.4.24.4	EC 3.4.24.25 aeromonolysin, EC 3.4.24.26 pseudolysin, EC 3.4.24.27 thermolysin, EC 3.4.24.28 bacillolysin, EC 3.4.24.29 aureolysin, EC 3.4.24.30 coccolysin, EC 3.4.24.31 mycolysin, EC 3.4.24.32 β-lytic metalloendopeptidase, EC 3.4.24.39 deuterolysin, EC 3.4.24.40 serralysin

Current enzyme entry in the Code	Updated enzyme reclassification and names based on IUBMB review of peptidases
serine proteinase EC 3.4.21.14	EC 3.4.21.62 subtilisin; EC 3.4.21.63 oryzin; EC 3.4.21.64 endopeptidase K; EC 3.4.21.65 thermomycolin; and EC 3.4.21.66 endopeptidase So

We propose to replace the entries for the above four enzymes, with those suggested to us from industry, provided they are from the already approved sources as listed in the Code. This is briefly discussed in Table 4 above and in more detail at attachment 3. These changes may lead to some already approved microbial source organisms being deleted.

Initial changes have been suggested based on advice received from the industry members of the External Advisory Group. Enzymes from the reclassified group that are not produced from already specifically permitted sources will need to be separately approved by FSANZ.

FSANZ seeks additional comment from industry to confirm which of the updated enzymes (and microbial source) are being used in place of –
bromelain EC 3.4.22.4
carboxyl proteinase EC 3.4.23.6,
metalloproteinase EC 3.4.24.4, and
serine proteinase EC 3.4.21.14.

5.3.4 Updating source organism nomenclature

Comments received from industry stakeholders also included a number of suggestions to update the nomenclature of the source micro-organisms approved for this purpose.

FSANZ has therefore acknowledged that *Bacillus stearothermophilus* is the former name for *Geobacillus stearothermophilus* and all relevant changes to the Code have been proposed.

FSANZ agrees with industry advice that since 1987 *Bacillus amyloliquefaciens* is no longer a strain of the species of *Bacillus subtilis*, but a species in its own right. Consequently the relevant editorial comment will be deleted. FSANZ, with the assistance of industry EAG members, has proposed that a number of entries for *B. subtilis* should read *Bacillus amyloliquefaciens.*

FSANZ seeks additional comment from industry to confirm which of their enzymes are sourced from *Bacillus amyloliquefaciens* and which are sourced from *Bacillus subtilis.*

5.4 Generic approvals

It was suggested that FSANZ should consider a generic approval for any source microbial organism to contain inserted genes from any other already approved source microbial organism. In addition, some submitters support inclusion of enzymes that the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) have previously classified as safe, and similar nomenclature as far as possible.

5.4.1 Evaluation

Updating the enzyme permissions in the processing aids standard by reference to the AMFEP list would not be satisfactory because the AMFEP list is simply a list of commercially available enzymes. The enzymes have not necessarily undergone a formal safety assessment, although AMFEP do observe the recommendations of the JECFA 'General Considerations and Specifications for Enzyme Preparations from GMOs'. In addition, where an enzyme is already permitted, but a new source was requested to be included in the standard, it must be demonstrated that the new source had a safe history of use for the production of enzymes used in food.

This Proposal is not a vehicle to give approvals for new, currently non-approved enzymes. Applicants would still need to make applications to request permissions to use new enzymes in the Code.

5.5 Other issues

One submitter considered that enzymes should be reclassified as ingredients requiring labelling or, that their GM status be declared on the label to enable consumers to make an informed choice.

5.5.1 Evaluation

Enzymes listed in clauses 15, 16 and 17 to Standard 1.3.3 are specifically permitted for use in food as processing aids. Clause 3(d) of Standard 1.2.4 – Labelling of Ingredients specifically exempts processing aids from being listed in a statement of ingredients. Reclassification of enzymes from processing aids to ingredients (that require labelling) is outside the scope of this review. However, FSANZ is satisfied that where enzymes are approved for use as processing aids, they continue to be exempt from listing in a statement of ingredients as it appears on a label.

Under Standard 1.5.2-Food Produced Using Gene Technology if a food, food ingredient, additive or **processing aid** contains novel DNA or protein that has come from an approved GM food, it must be labelled with the words 'genetically modified'. The statement 'genetically modified' must be used in conjunction with the name of the food or in association with the specific ingredient in the ingredient list. If the food is unpackaged then the information that otherwise would have been on the package must be displayed on or in connection with the display of the food.

In the case of enzymes produced from genetically modified micro-organisms the enzyme is not a novel protein since it is identical to other enzymes sourced from non-genetically derived sources. The refinement process for the enzyme preparation removes all the source organism from the preparation so there is no novel DNA in the enzyme preparation. Therefore small amounts of enzymes (inactivated or not) from a genetically modified source remaining in food do not require labelling under the gene technology labelling requirements. This is the case for all enzymes sourced from a genetically modified micro-organism (of which there are a number approved in the Code).

6. Regulatory Options

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

The following two regulatory options are available for this Proposal.

Option 1. Maintain the status quo and not amend clauses 15, 16 and 17 of Standard 1.3.3.

Option 2. Review clauses 15, 16 and 17 of Standard 1.3.3 and make amendments to the Code as required.

7. Impact Analysis

7.1 Affected Parties

The affected parties to this Proposal are:

- food manufacturers of every category who use enzymes in manufacturing and packaging their food products in Australia and New Zealand;
- consumers of food;
- manufacturers and suppliers of food enzymes; and
- Australian, State, Territory and New Zealand government enforcement agencies.

7.2 Impact Analysis

This Proposal is seeking to make amendments to review specific clauses of Standard 1.3.3 in relation to safety of currently approved enzymes, nomenclature used for enzymes and source organisms, and consider enzymes not currently used in Australia and New Zealand. However, it is not expected that there should be any major costs or impacts to food manufacturers, consumers or regulatory agencies. It is anticipated that there should be benefits to consumers that the safety of currently approved enzymes has been confirmed.

7.2.1 Option 1 Status quo

7.2.1.1 Industry

Because this option will not change the Code there should not be any immediate impact on industry. It does however deny industry an improved level of clarification that will be found in option 2.

7.2.1.2 Consumers

There is no immediate effect on consumers of this option.

7.2.1.3 Government

The impact of this option denies regulatory agencies of improved clarity of Standard 1.3.3 as being suggested in option 2.

7.2.2 Option 2 Amend the Code

7.2.2.1 Industry

It is not expected that there should be any costs or detrimental effects on industry because of the outcomes of amending Standard 1.3.3 to review enzyme permissions.

This Draft Assessment Report containing the proposed amendments will be circulated for a round of public comment so if any proposed amendments will cause unnecessary or unintended imposts on industry, FSANZ will be made aware of this and can assess these costs verses the proposed benefits from the change. Any such submissions will be assessed as part of the Final Assessment where final draft variations will be made.

7.2.2.2 Consumers

The advantage for consumers is that the safety of currently approved processing aids has been assessed with the most recent technical information. There is no other immediate effect on consumers of this option.

7.2.2.3 Government

The impacts of this option should be minimal for regulatory agencies. One advantage is that inconsistencies within the Code, specifically different nomenclature for enzymes will have been removed, so eliminating some unnecessary confusion and enquiries.

Also this Proposal will not be approving new enzymes, so there should be little impacts on regulatory agencies.

8. Consultation

8.1 Public consultation

The Initial Assessment report for P276 was circulated for a round of public comment from 17 December 2003 till 25 February 2004. Ten submissions were received, nine supporting the review and one raised issues which were outside of the review mandate. A summary of submissions is contained in **Attachment 4**. Issues raised in these submissions are discussed in section 5 above.

FSANZ seeks further advice as part of this review. The questions and issues upon which specific advice is sought are listed below.

- Are there any obsolete enzymes which are no longer used, or likely to ever be used again in the food industry, in either Australia or New Zealand? Possible use internationally must also be considered since our trading partners export food products to both Australia and New Zealand and these products must also meet the requirements of the Code. If none are identified, then FSANZ will retain the current permissions.

- From the list of permitted enzymes, which enzymes are sourced from Bacillus amyloliquefaciens and which are sourced from Bacillus subtilis, as B. amyloliquefaciens is no longer considered a strain of B. subtilis but a species in its own right.

- Confirmation, especially from industry, is being sought on the suggested replacement entries for the following four enzymes below.
  Bromelain;
  Carboxyl proteinase;
  Metalloproteinase; and
  Serine proteinase.
  Which microbes are used to source the suggested replacement enzymes?

- Are there any adverse implications for industry associated with the proposed amendment to the entry for 6-phytase EC 3.1.3.26 to read 4-phytase EC 3.1.3.26?

8.2 External Advisory Group

The FSANZ Board agreed to the establishment of a committee and to the appointment of appropriate qualified and skilled people to an External Advisory Group (EAG) to provide advice to FSANZ to assist with completing the review of processing aids. This EAG has been drawn from experts from industry groups, regulatory agencies, academic and consumer groups with knowledge and expertise in food enzymes and their regulation.

The EAG met (with some members being linked in via teleconference) in Sydney in May 2004. This meeting confirmed the terms of reference for the group and assisted in addressing issues received from submissions as well as providing expert advice on proposed amendments to the Standard. Further meetings or teleconferences will be conducted to address issues and proposed amendments, as well as communications via email on specific issues, as required.

The terms of reference of the EAG is contained in **Attachment 5**.

8.3 World Trade Organization (WTO)

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

There are not any relevant international standards for processing aids and amending the Code to update and improve Standard 1.3.3 – Processing Aids is unlikely to have a significant effect on international trade.

However, since FSANZ is seeking input in relation to international usage of enzymes (in particular in relation to possible deletions of permissions for obsolete enzymes). Therefore, a notification will be made to the agencies responsible in accordance with Australia's and New Zealand's obligations under the WTO Technical Barrier to Trade (TBT).

9. The Decision

Preferred Approach

FSANZ has reviewed clauses 15, 16 and 17 of Standard 1.3.3 – Processing Aids and has proposed a number of draft variations. These are proposed to ensure public health and safety, correct errors, remove duplications and anomalies, ensure consistency and improve the function of the Standard.

The draft variations to Standard 1.3.3 – Enzyme Processing Aids of the Code are recommended for the following reasons:

- The proposed amendments are consistent with the protection of public health and safety since.

- The proposed amendments also ensure consistency within the Code and improved consistency, as far as is possible, with other international food standards.

- The proposed amendments have included information and submissions on issues received, as well as advice from an Expert Advisory Group, made up of experts external to FSANZ.

- There will not be any expected added costs to food manufacturers, consumers or regulatory agencies arising from these proposed amendments.

- There are no other alternatives that are more cost effective than the proposed amendments to the Code.

ATTACHMENTS

1. Draft variations to the *Australia New Zealand Food Standards Code*
2. Safety assessment report
3. Review of nomenclature of currently permitted enzymes and suggested amendments
4. Summary of Submissions
5. Terms of reference of the External Advisory Group

Attachment 1

Draft variations to the *Australia New Zealand Food Standards Code*

To commence: on gazettal

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

[1.1] *omitting from clause 1 the definition of* EC [number]*, substituting –*

EC number (Enzyme Commission number) means the number which the Enzyme Commission uses to classify the principal enzyme activity.

[1.2] *omitting the* Table to clause 15, *substituting –*

Enzyme	Source
Lipase, triacylglycerol EC 3.1.1.3	Bovine stomach; salivary glands or forestomach of calf, kid or lamb; porcine or bovine pancreas
Pepsin EC 3.4.23.1	Bovine or porcine stomach
Phospholipase A_2 EC 3.1.1.4	Porcine pancreas
Thrombin EC 3.4.21.5	Bovine or porcine blood
Trypsin EC 3.4.21.4	Porcine or bovine pancreas

[1.3] *omitting the* Table to clause 16, *substituting –*

Enzyme	Source
α–Amylase EC 3.2.1.1	Malted cereals
β-Amylase EC 3.2.1.2	Sweet potato (*Ipomoea batatas)* Malted cereals
Actinidin EC 3.4.22.12	Kiwifruit (*Actinidia deliciosa*)
Bromelain, stem EC 3.4.22.32	Pineapple stem (*Ananas comosus)*
Ficin EC 3.4.22.3	*Ficus* spp.
Papain EC 3.4.22.2	*Carica papaya*

[1.4] *omitting the* Table to clause 17, *substituting –*

Enzyme	Source
α–Acetolactate decarboxylase EC 4.1.1.5	*Bacillus subtilis* *Bacillus subtilis*, containing the gene for α–Acetolactate decarboxylase isolated from *Bacillus brevis*
α–Amylase EC 3.2.1.1	*Aspergillus niger* *Aspergillus oryzae* *Bacillus amyloliquefaciens* *Bacillus licheniformis* *Bacillus licheniformis*, containing the gene for α-Amylase isolated from *Geobacillus stearothermophilus* *Bacillus subtilis*, containing the gene for α-Amylase isolated from *Geobacillus stearothermophilus* *Geobacillus stearothermophilus*
β-Amylase EC 3.2.1.2	*Bacillus subtilis*
α–Arabinofuranosidase EC 3.2.1.55	*Aspergillus niger*
Aspergillopepsin I EC 3.4.23.18	*Aspergillus niger* *Aspergillus oryzae*
Aspergillopepsin II EC 3.4.23.19	*Aspergillus niger*
Bacillolysin EC 3.4.24.28	*Bacillus amyloliquefaciens* *Bacillus subtilis*
Carboxylesterase EC 3.1.1.1	*Rhizomucor miehei*
Catalase EC 1.11.1.6	*Aspergillus niger* *Micrococcus luteus*
Cellulase EC 3.2.1.4	*Aspergillus niger* *Trichoderma reesei* *Trichoderma viride*
Chymosin EC 3.4.23.4	*Aspergillus niger* *Escherichia coli* K-12 strain GE81 *Kluyveromyces lactis*
Cyclodextrin glucanotransferase EC 2.4.1.19	*Paenibacillus macerans*
Deuterolysin EC 3.4.24.39	*Aspergillus oryzae*
Dextranase EC 3.2.1.11	*Chaetomium gracile* *Penicillium lilacinum*
Endo-arabanase EC 3.2.1.99	*Aspergillus niger*
Endo-1,3-β-xylanase EC 3.2.1.32	*Humicola insolens*
Endo-1,4- β-xylanase EC 3.2.1.8	*Aspergillus niger* *Aspergillus oryzae* *Aspergillus oryzae*, containing the gene for Endo-1,4-β-xylanase isolated from *Aspergillus aculeatus* *Aspergillus oryzae*, containing the gene for Endo-1,4-β-xylanase isolated from *Thermomyces lanuginosus* *Bacillus amyloliquefaciens* *Humicola insolens* *Trichoderma reesei*

Enzyme	Source
Endo-1,4- β-mannanase EC 3.2.1.78	*Aspergillus niger* *Bacillus amyloliquefaciens* *Trichoderma reesei*
α-Galactosidase EC 3.2.1.22	*Aspergillus niger*
β-Galactosidase EC 3.2.1.23	*Aspergillus niger* *Aspergillus oryzae* *Saccharomyces fragilis* *Saccharomyces lactis*
Glucan 1,3-β-glucosidase EC 3.2.1.58	*Trichoderma harzianum*
β-Glucanase EC 3.2.1.6	*Aspergillus niger* *Aspergillus oryzae* *Bacillus subtilis* *Disporotrichum dimorphosporum* *Humicola insolens* *Talaromyces emersonii* *Trichoderma reesei*
Glucoamylase EC 3.2.1.3	*Aspergillus niger* *Aspergillus oryzae* *Rhizopus delemar* *Rhizopus oryzae* *Rhizopus niveus*
Glucose oxidase EC 1.1.3.4	*Aspergillus niger* *Aspergillus oryzae,* containing the gene for glucose oxidase isolated from *Aspergillus niger*
α-Glucosidase EC 3.2.1.20	*Aspergillus oryzae* *Aspergillus niger*
β-Glucosidase EC 3.2.1.21	*Aspergillus niger*
Hexose oxidase EC 1.1.3.5	*Hansenula polymorpha,* containing the gene for Hexose oxidase isolated from *Chondrus crispus*
Inulinase EC 3.2.1.7	*Aspergillus niger*
Invertase EC 3.2.1.26	*Saccharomyces cerevisiae*
Leucyl aminopeptidase EC 3.4.11.1	*Lactococcoccus lactis* *Aspergillus oryzae*
Lipase, monoacylglycerol EC 3.1.1.23	*Penicillium camembertii*
Lipase, triacylglycerol EC 3.1.1.3	*Aspergillus niger* *Aspergillus oryzae* *Aspergillus oryzae*, containing the gene for Lipase, triacylglycerol isolated from *Fusarium oxysporum* *Aspergillus oryzae*, containing the gene for Lipase, triacylglycerol isolated from *Humicola lanuginosa* *Aspergillus oryzae*, containing the gene for Lipase, triacylglycerol isolated from *Rhizomucor miehei* *Candida rugosa* *Mucor javanicus* *Penicillium roquefortii* *Rhizopus arrhizus* *Rhizomucor miehei* *Rhizophus niveus* *Rhizophus oryzae*
Lysophospholipase EC 3.1.1.5	*Aspergillus niger*

Enzyme	Source
Maltogenic α-amylase EC 3.2.1.133	*Bacillus subtilis* containing the gene for maltogenic amylase isolated from *Geobacillus stearothermophilus*
Mucorpepsin EC 3.4.23.23	*Aspergillus oryzae* *Aspergillus oryzae*, containing the gene for Aspartic proteinase isolated from *Rhizomucor meihei* *Rhizomucor meihei* *Cryphonectria parasitica*
Pectin lyase EC 4.2.2.10	*Aspergillus niger*
Pectinesterase EC 3.1.1.11	*Aspergillus niger* *Aspergillus oryzae,* containing the gene for pectinesterase isolated from *Aspergillus aculeatus*
Phospholipase A_1 EC 3.1.1.32	*Aspergillus oryzae,* containing the gene for phospholipase A_1 isolated from *Fusarium venenatum*
Phospholipase A_2 EC 3.1.1.4	*Streptomyces violaceoruber*
3-Phytase EC 3.1.3.8	*Aspergillus niger*
4-Phytase EC 3.1.3.26	*Aspergillus oryzae*, containing the gene for 4-phytase isolated from *Peniophora lycii*
Polygalacturonase EC 3.2.1.15	*Aspergillus niger* *Aspergillus oryzae* *Trichoderma reesei*
Pullulanase EC 3.2.1.41	*Bacillus acidopullulyticus* *Bacillus licheniformis* *Bacillus subtilis* *Klebsiella pneumoniae*
Subtilisin EC 3.4.21.62	*Bacillus licheniformis* *Bacillus halodurans*
Thermolysin EC 3.4.24.27	*Aspergillus oryzae*
Transglucosidase EC 2.4.1.24	*Aspergillus niger*
Transglutaminase EC 2.3.2.13	*Streptomyces mobaraense*
Urease EC 3.5.1.5	*Lactobacillus fermentum*
Xylose isomerase EC 5.3.1.5	*Actinoplanes missouriensis* *Bacillus coagulans* *Microbacterium arborescens* *Streptomyces olivaceus* *Streptomyces olivochromogenes* *Streptomyces murinus* *Streptomyces rubiginosus*

[1.5] *omitting from the* Editorial note*, immediately following the* Table to clause 17 –

Bacillus subtilis covers the strain known under the name *Bacillus amyloliquefaciens*.

[1.6] *inserting into the* Editorial note*, immediately following the* Table to clause 17 –

Bacillus stearothermophilus is the former name for *Geobacillus stearothermophilus*.

Attachment 2

Safety Assessment of Certain Enzyme Processing Aids

SUMMARY AND CONCLUSIONS

A total of seven enzyme processing aids have been evaluated for their safety (Table 1). The enzymes were selected for evaluation on the basis that they had recently been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The safety of each of the selected enzyme processing aid was reviewed based on the evaluation undertaken by JECFA.

The evaluation was based on consideration of the pathogenicity and toxicity of the source organism, oral toxicity and genotoxicity of the enzyme preparation, as well as any genetic modification that had been undertaken in the development of the production strain.

All the enzymes evaluated were determined to have low oral toxicity and were thus considered to raise no toxicological or other safety concerns.

Table 1: Summary of safety assessment conclusions

Enzyme processing aid	Safety assessment conclusions
α-Acetolactate decarboxylase (EC 4.1.1.5) from *Bacillus brevis* expressed in *Bacillus subtilis*	No toxicological concerns
α-Amylase (EC 3.2.1.1) from *Bacillus licheniformis* expressed in *Bacillus licheniformis*	No toxicological concerns
Hexose oxidase (EC 1.1.3.5) from *Chondrus crispus* expressed in *Hansenula polymorpha*	No toxicological concerns
Invertase (EC 3.2.1.26) from *Saccharomyces cerevisiae*	No toxicological concerns
Maltogenic amylase (EC 3.2.1.133) from *Bacillus stearothermophilus* expressed in *Bacillus subtilis*	No toxicological concerns
Xylanases (EC 3.2.1.8) from B*acillus subtilis* expressed in *Bacillus subtilis*	No toxicological concerns
Mixed β-glucanase (EC 3.2.1.6) and xylanase (EC 3.2.1.8) enzyme preparation, produced by a strain of *Humicola insolens*	No toxicological concerns

1. INTRODUCTION

1.1 Historical background

A proposal for the development of a standard to regulate the use of processing aids, including enzyme processing aids (Proposal P86) was raised in 1995 and resulted in the development of Standard A16, which was gazetted in the former *Australian Food Standards Code* in April 1996. The standard was developed for Australia only.

Standard A16 was subsequently reviewed under Proposal P188, as part of the review of the *Australian Food Standards Code*, resulting in the development of Standard 1.3.3 of the *Australia New Zealand Food Standards Code*. The objective of P188 was to update Standard A16 to recognise current practices in Australia and to take account of New Zealand requirements from the *New Zealand Food Regulations 1984*, in order to implement a joint Code with New Zealand. As Standard A16 had only recently been included in the *Australian Food Standards Code,* and had been based on a toxicology evaluation, a detailed review (including a toxicology report) was not considered necessary.

The toxicological evaluation undertaken for P86 noted that the majority of processing aids are either not present in the final food or present at such low levels that they do not constitute a concern for public health and safety. A number of processing aids were found to leave residues in food or to have a demonstrated toxicity and these were assessed to ensure that the levels present in food were safe. The assessment also provided the scientific justifications for maximum residue levels set for processing aids, if they were warranted for the protection of public health and safety.

In the case of enzymes used in food processing, the main toxicological considerations relate to possible contaminants in the enzyme preparations, as typically the enzymes themselves are non-toxic. Enzyme processing aids are also 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. JECFA (FAO, 2001) and the Food Chemical Codex (Food Chemical Codex, 2004) have recommended specifications for food-grade enzymes. Enzymes used in food for sale in Australia and New Zealand need to comply with Standard 1.3.4 – Identity and Purity.

1.2 Criteria used to select enzyme processing aids for assessment

The following criteria have been used to select the enzyme processing aids for assessment under this Proposal:

(i) the enzyme has been (re)evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), or another scientific agency[2] since 1995; or
(ii) the substance has been identified by FSANZ, or other parties, as of potential toxicological concern.

2. SAFETY ASSESSMENT

2.1 α-Acetolactate decarboxylase from *Bacillus brevis* expressed in *B. subtilis*

Background

α-Acetolactate decarboxylase (EC 4.1.1.5) is produced by submerged fermentation of *B. subtilis* carrying the gene coding for α-acetolactate decarboxylase (A*ldB*), which was isolated from *B. brevis*. Construction of the recombinant *B. subtilis* strain, containing the *AldB* gene was done using standard recombinant-DNA techniques.

[2] e.g. National Industrial Chemicals Notification and Assessment Scheme (NICNAS), National Health and Medical Research Council (NHMRC), European Scientific Committee on Food (SCF), European Food Safety Authority (EFSA), the United Stated Environmental Protection Agency (US EPA), Agency for Toxic Substances and Disease Registry (ATSDR), International Agency for Research on Cancer (IARC), and the Environmental Health Criteria (EHC)

α-Acetolactate decarboxylase is used as a processing aid in the brewing and alcohol industries where it is used to avoid formation of the unpleasant tasting α-diacetyl from α-acetolactate during fermentation. In the traditional brewing process, the α-diacetyl formed from α-acetolactate is further reduced to acetoin over a 2- to 4-week maturation period. Alpha-acetolactate decarboxylase causes direct decarboxylation of α-acetolactate to acetoin, thus avoiding the need for this maturation period. The enzyme can similarly be used in the fermentation of alcohol, where diacetyl is otherwise formed and requires a maturation step before distillation.

Summary of available safety information

JECFA evaluated α-acetolactate decarboxylase from *B. brevis* expressed in *B. subtilis* in 1998, examining host and donor strain pathogenicity, acute and short term toxicity of the enzyme preparation, as well as genotoxicity (WHO, 1998a). No long-term studies were available. On the basis of its evaluation, JECFA concluded that α-acetolactate decarboxylase is an enzyme of low toxicity and that no further studies are required to assess its safety. JECFA established a temporary ADI 'not specified' for α-acetolactate decarboxylase from the recombinant strain of *B. subtilis* when the preparation is used in accordance with good manufacturing practice (GMP). A temporary ADI was allocated because the specifications are temporary.

Construction of the production strain

Construction of the genetically modified *B. subtilis* strain, UW227, which contains the *AldB* gene was done using several steps involving the isolation and cloning of the *AldB* gene from *B. brevis* and its subsequent introduction, via plasmid transformation, into *B. subtilis*. The kanamycin resistance gene, which was originally present in the plasmid containing the *AldB* gene, was removed in the final strain construction steps.

Host and donor strain pathogenicity

The pathogenicity of both the host and donor organisms was evaluated by investigating cases of human infections as well as a consideration of the history of use of these organisms in food. A specific study on the pathogenicity of the host organism, *B. subtilis*, was also undertaken in mice.

The host organism, *B. subtilis,* is considered to be a non-pathogenic species, and has a history of safe use in food enzyme manufacturing. Similarly, in an examination of reviews dealing with infections caused by *Bacillus* spp., the donor organism, *B. brevis,* was found in only one report to have caused infection (in one patient). No other cases of infection by *B. brevis* were noted in these reviews. *B. brevis* is therefore regarded as a non-pathogenic organism.

In a study to investigate the pathogenicity of four *B. subtilis* strains involved in either the construction of the α-acetolactate decarboxylase-producing strain or in producing α-acetolactate decarboxylase, three separate groups of five mice each were treated intraperitoneally with a particular strain of *B. subtilis* at varying dose levels between 2-7.6 × 10^5 and 2-7.6 × 10^8 cells/kg bw. A control group received a buffer solution. The mice were observed for 30 minutes after dosing for clinical symptoms associated with treatment and then daily for 14 days. At the end of the 14-day period, all mice were sacrificed and a macroscopic pathological examination performed.

There were no clinical symptoms related to treatment and no pathological changes noted at the end of the study that could be associated with treatment.

Short-term toxicity

Two forms of α-acetolactate decarboxylase were used for the toxicity studies – an unstabilised form, referred to as ALDC, and a gluteraldehyde-stabilised form, referred to as d-ALDC. The gluteraldehyde-stabilised form is the form used in the final commercial product.

Both 14-day and 13-week feeding studies were undertaken in rats at dietary levels equivalent to 2500 mg/kg bodyweight per day (14-day study) or 500 mg/kg bw/day (13-week study) using both ALDC and d-ALDC. No clinical signs of toxicity were observed during both studies and there were also no treatment-related macroscopic or microscopic pathological changes and no significant toxicological changes at any of the dose levels tested.

Genotoxicity

In the genotoxicity studies, negative results were obtained with both ALDC and d-ALDC in both the bacterial and mammalian gene mutation assays and in a chromosome aberration assay in human lymphocytes.

Evaluation and conclusion

α-Acetolactate decarboxylase from *B. brevis* expressed in *B. subtilis* is an enzyme of low oral toxicity and both the source and donor organisms are non-pathogenic to humans. There are **no toxicological or other safety concerns** with the use of α-acetolactate decarboxylase from *B. brevis* expressed in *B. subtilis* as a processing aid.

2.2 α-Amylase from Bacillus licheniformis, expressed in B. licheniformis

Background

The α-amylase (EC 3.2.1.1) enzyme preparation contains the LE399 α-amylase from a genetically modified strain of *Bacillus licheniformis*. The enzyme is thermostable and active at a relatively low pH and low calcium concentration. These characteristics make the enzyme particularly suitable for use in starch hydrolysis conducted at high temperatures, for example, for the liquefaction of starch used in the production of nutritive sweeteners.

The LE399 α-amylase is produced by pure culture fermentation of a strain of *B. licheniformis*. The enzyme is subsequently partially purified and concentrated, resulting in a liquid enzyme concentrate (LEC). In the final preparation, the LEC is stabilized and standardized and formulated with methionine, sodium chloride, and glucose and sucrose.

α-Amylases break down starch into soluble dextrins and oligosaccharides via endohydrolysis of 1,4-α-glucosidic linkages in amylose and amylopectin. This results in a rapid reduction of the viscosity of gelatinized starch. The LE399 α-amylase can operate at lower pH and lower concentrations of calcium ions than conventional heat-stable α-amylases.

Summary of available safety information

JECFA evaluated α-amylase from a genetically modified strain of *B. licheniformis* in 2003, examining the methods used to construct the production strain, short term toxicity of the enzyme preparation, as well as genotoxicity (WHO, 2004c). The Committee allocated an ADI 'not specified' to α-amylase from this source, used in the applications specified and in accordance with good manufacturing practice. The Committee concluded that no residual LE399 α-amylase is expected to be present in food processed using this enzyme preparation. The α-amylase preparation is intended for use in starch liquefaction in the production of sweetener syrups, alcoholic beverages and beer. The absence of the α-amylase protein in the final (purified) sweetener syrup has been confirmed experimentally. In the spirits industry, no LE399 α-amylase or other organic solids are expected to be carried over to the final product because ethanol is removed by distillation from the fermentation mash containing the enzyme preparation. In the brewing of beer, the enzyme preparation is added during the mashing process and is denatured and inactivated during the subsequent wortboiling stage. The beer filtration process is likely to remove the denatured enzymes along with other insoluble materials.

Construction of the production strain

The LE399 α-amylase protein was developed by changing four amino acids in the polypeptide chain of another thermostable α-amylase; the Termamyl α-amylase. The LE399 α-amylase gene was then introduced into the host strain SJ5550.

The host strain was developed from a parent strain DN2717, a derivative of a natural *B. licheniformis* isolate. The DN2717 strain was genetically modified to inactivate the following native genes: the *apr* gene encoding the 'Alkalase' protease; the *amyL* gene encoding the Termamyl α-amylase; the *xyl* gene encoding xylose isomerase; and the *gnt* gene encoding gluconate permease. The inactivated *amyL*, *xyl*, and *gnt* genes were replaced with three copies of the LE399 α-amylase gene. In a separate step, the gene encoding C-component protease was deleted. The resulting strain was designated as MOL2083 and used as a production strain. The aim of these genetic modifications was to prevent the synthesis of proteases that might hydrolyse the LE399 α-amylase, and to avoid the production of the Termamyl α-amylase.

The genetic material introduced into the production strain has been well characterized and does not contain any sequences that would encode for proteins resulting in the production of toxic or undesirable substances. The LE399 α-amylase gene is stably integrated into the *B. licheniformis* chromosome. The production strain, which is both non-pathogenic and non-toxigenic, does not contain genes encoding proteins that inactivate antibiotics.

Short-term toxicity

In a 13-week study in rats (10 male, 10 female), no significant treatment-related effects were seen when the α-amylase enzyme preparation was administered in water by oral gavage at doses of up to and including 1020 mg/kg bw per day, expressed as TOS (total organic solids from the fermentation; mainly protein and carbohydrate components). The highest dose tested was considered to be the no-observed-effect-level (NOEL).

Genotoxicity

Two genotoxicity studies were done with the α-amylase enzyme preparation. The enzyme preparation was not mutagenic in an assay for mutagenicity in bacteria *in vitro* and was not clastogenic in an assay for chromosomal aberrations in mammalian cells *in vitro*.

Other studies

The LE399 α-amylase was assessed for potential allergenicity by amino acid sequence comparison with known allergens listed in publicly available protein databases. No immunologically significant sequence similarity was detected.

Evaluation and conclusion

α-Amylase from *B. licheniformis* is an enzyme of low oral toxicity and the production organism is both non-pathogenic and non-toxigenic to humans. There are **no toxicological or other safety concerns** with the use of α-amylase from *B. licheniformis* as a processing aid.

2.3 Hexose oxidase from *Chondrus crispus* expressed in *Hansenula polymorpha*

Background

Hexose oxidase (EC 1.1.3.5) catalyses the oxidation of C6 sugars to their corresponding lactones, with the concomitant formation of hydrogen peroxide and is used as an alternative to glucose oxidase in the baking industry to strengthen dough and, in a similar way, in the pasta and noodle industries to produce a firmer structure. Hexose oxidase is also used in foods for which the browning Maillard reactions that normally occur with heating are not desirable, and in cheese and tofu manufacture to improve curd formation. Hexose oxidase has the highest affinity for D-glucose and D-galactose.

The enzyme is produced by submerged fermentation of a pure culture of the genetically modified strain of the yeast *Hansenula polymorpha*, containing the hexose oxidase gene derived from the red alga *Chondrus crispus*. *C. crispus* has a long history of use in food in Asia and is not known to be either pathogenic or toxigenic.

The enzyme is produced intracellularly and upon cell disruption with lauryl trimethyl ammonium bromide (LTAB) is released into the fermentation broth and subsequently purified using filtration steps. Owing to carry over of LTAB into the enzyme preparation, it is possible that small amounts of this quaternary ammonium compound might be present in the final food. Enzyme activity is expressed in hexose oxidase units (HOXU).

Summary of available safety information

Hexose oxidase from *C. crispus* expressed in *H. polymorpha* was evaluated by FSANZ in 2003 (FSANZ, 2003) and also by JECFA in 2004 (WHO, 2004a). Data was evaluated on the construction of the production strain, acute and short-term toxicity, as well as genotoxicity. No long-term studies were available.

The Committee allocated an ADI 'not specified' to hexose oxidase from *H. polymorpha* when used in the applications specified and in accordance with good laboratory practice. The enzyme preparation conforms to the *General specifications for enzyme preparations in food processing* (Annex 1)(FAO 2001). The Committee concluded that the presence of LTAB at the concentrations observed in the enzyme preparation would not pose a safety concern to consumers. The enzyme is typically denatured during heat treatment, and is no longer active in the final food product as eaten.

Construction of the production strain

A synthetic hexose oxidase gene was constructed, based on hexose oxidase from *C. crispus*, in order to optimise protein expression in yeast. The hexose oxidase expressed from the synthetic gene is identical in amino acid sequence to the native *C. crispus* hexose oxidase. The synthetic hexose oxidase gene was combined with regulatory sequences for expression in yeast and transferred to *H. polymorpha* via plasmid transformation. No antibiotic resistance genes were transferred in this process. The introduced DNA in *H. polymorpha* is well characterised and would not result in the production of any toxic or undesirable substances. The production strain is stable with respect to the introduced DNA.

Acute and short-term toxicity

Studies were done using water-soluble turbid liquid concentrates produced from fermentation of *H. polymorpha* carrying the synthetic hexose oxidase gene.

These enzyme preparations were not acutely toxic when tested in rats, giving an LD_{50} of >2000 mg/kg body weight.

In a range finding study in rats (5 male, 5 female), doses equivalent to 0, 500, 1250 or 5000 HOXU/kg body weight/day were administered by gavage for 2 weeks. No treatment related adverse effects were observed up to and including the highest dose level tested.

Groups of rats (10 male, 10 female) were administered hexose oxidase at a dose equivalent to 0, 500, 1250 or 5000 HOXU/kg bodyweight/day by gavage for 13 weeks. The enzyme preparation also contained LTAB. No treatment related adverse effects were observed. The NOEL for this study was 5000 HOXU, equivalent to an intake of total organic solids of 955 mg/kg bodyweight/day. This highest dose was also equivalent to an exposure to LTAB at 11.3 mg/kg bodyweight/day.

Genotoxicity

The hexose oxidase preparation, containing LTAB, was evaluated for genotoxicity in vitro and was found to be non-mutagenic in bacterial cells and non-clastogenic in an assay for chromosomal aberrations in mammalian cells.

Evaluation and conclusion

Hexose oxidase from *C. crispus* expressed in *H. polymorpha* is an enzyme of low oral toxicity and the donor and production organisms are both non-pathogenic and non-toxigenic to humans. There are **no toxicological or other safety concerns** with the use of hexose oxidase from *C. crispus* expressed in *H. polymorpha* as a processing aid.

2.4 Invertase from Saccharomyces cerevisiae

Background

Invertase (EC 3.2.1.26) catalyses the hydrolysis of sucrose into glucose and fructose. It is used in the production of confectionery and in the ethanol industry. Invertase is produced by a wide range of organisms, such as *Neurospora crassa*, *Candida utilis*, *Aspergillus niger* and *Saccharomyces cerevisiae*. *S. cerevisiae* shows the greatest ability to secrete invertase.

Summary of available safety information

Invertase from *S. cerevisiae* was evaluated by JECFA in 2001 (WHO, 2002). No biological data were available. *S. cerevisiae* has a well-established history of use in fermented foods, including bread, alcoholic beverages, some milk products and cocoa. In line with the general principles outlined in Principles for the safety assessment of food additives and contaminants in food (WHO, 1987), invertase from *S. cerevisiae* that meets the specifications was considered to be acceptable, as *S. cerevisiae* is commonly used in the preparation of food. Its use should be limited by good manufacturing practice.

Evaluation and conclusion

Based on the long history of use of invertase from *S. cerevisiae* in food, there are **no toxicological or other safety concerns** with its use as an enzyme processing aid.

2.5 Maltogenic amylase from Bacillus stearothermophilus expressed in Bacillus subtilis

Background

Formulations of maltogenic amylase (E.C.3.2.1.133) are used in the baking and starch industry. It is an exo-acting maltogenic amylase enzyme and catalyses the hydrolysis of α-1,4-glucosidic linkages in amylose, amylopectin and related glucose polymers. Maltose units are successively removed from the non-reducing end of the polymer chain until the molecule is degraded or, in the case of amylopectin, a branch-point is reached.

The preparations of maltogenic amylase, which are the subject of this assessment, are produced by submerged fermentation of a strain of *B subtilis,* which has been genetically modified to contain the *amy*M gene from *B. stearothermophilus* coding for maltogenic amylase.

Summary of available safety information

Maltogenic amylase from *B. stearothermophilus* expressed in *B. subtilis* was evaluated by JECFA in 1998 (WHO, 1998b). The available data reviewed by the Committee included the genetic modification procedures used for constructing the production strain, characterization of the production organism, the fermentation process, short-term toxicity studies in animals, and genotoxicity studies.

The Committee allocated an ADI 'not specified' to maltogenic amylase derived from this genetically modified strain of *B. subtilis*. The Committee noted that well-documented non-pathogenic and non-toxicogenic strains of bacteria (*B. subtilis*, *Escherichia coli K12* and *B. stearothermophilus*) had been used in the genetic modification procedures. The Committee also concluded that the final construct should be regarded as a safe source of maltogenic amylase. The Committee also noted that the human intake of maltogenic amylase resulting from its intended use in the baking and starch industry would be low and that the material consumed would not be the active maltogenic amylase but a heated, denatured material.

Construction of the production strain

The maltogenic amylase gene *(amyM)* was isolated from the spore-forming bacterium *B. stearothermophilus* using standard cloning techniques and subsequently transferred, through several laboratory steps, into the production strain of *B. subtilis* (DN1413). The cloning vector used (pUB110) is well characterised and has been used for several years as a cloning vehicle for *B. subtilis*. The plasmid construct containing the *amyM* gene, pDN1413, was initially transferred to *B. subtilis* using standard transformation techniques and then subsequently became stably integrated into the chromosome of the production strain. Although the plasmid pDN1413 carries the kanamycin resistance gene it is considered unlikely to be transferred since it is stably integrated into the host genome and no residual plasmid DNA has been detected in the end product (limit of detection equivalent to 0.1 ng DNA/1 g enzyme). The entire DNA sequence of pDN1413 has been determined, confirming that Shiga-like toxins will not be produced.

Short-term toxicity studies

The product tested in the toxicological studies was a concentrated material (enzyme activity 35,900 maltogenic amylase units/g). It was produced according to the standard production process except that the formulation/standardization was omitted and the product was lyophilized.

Groups of 20 male and 20 female CD rats received the equivalent of 0, 390, 1200 or 4000 mg maltogenic amylase/kg bw/day for males and 0, 440, 1300 or 4300 mg maltogenic amylase/kg bw/day for females for 13 weeks.

No mortality was seen and no clinical signs due to treatment were observed. Ophthalmoscopy did not show any abnormalities. A slight decrease in food intake of males and females given the highest dose was seen, accompanied by a significantly decreased body weight gain. Haematology did not reveal treatment related abnormalities nor were there any treatment related changes of toxicological significance to clinical chemistry parameters. Organ weights revealed significantly lower absolute and relative thyroid weights in males at the highest dose tested. A significantly lower absolute lung weight was also observed in females at the highest dose level. Macroscopy and microscopy did not reveal any treatment related abnormalities. The NOAEL for this study was 1200 mg/kg bw/day (equivalent to 1.5% of the diet).

Genotoxicity studies

Both in vitro and in vivo genotoxicity studies have been conducted on the maltogenic amylase enzyme preparation. Negative results were obtained for gene mutation studies in both bacterial and mammalian cells and chromosomal aberration tests in vitro and vivo were consistently negative.

Evaluation and conclusion

Maltogenic amylase from *B. stearothermophilus* expressed in *B. subtilis* is an enzyme of low oral toxicity. Both the donor and production organisms are non-pathogenic and non-toxigenic to humans and can be regarded as a safe source of maltogenic amylase.

There are **no toxicological or other safety concerns** with the use of maltogenic amylase from *B. stearothermophilus* expressed in *B. subtilis* as a processing aid.

2.6 Xylanases from *Bacillus subtilis* expressed in *B. subtilis*

Background

Xylanase (EC 3.2.1.8) is an enzyme that catalyses the hydrolysis of xylans and arabinoxylans to mono- and oligosaccharides. Xylanase is used in the milling and baking industries, mainly to improve the dough. They may be used in yeast-raised or chemically leavened wheat and rye-based bakery products.

Three xylanases, designated BS1, BS2, and BS3, are derived from genetically modified strains of *B. subtilis*. Each xylanase is produced by pure culture fermentation of the respective production strain. Xylanases BS1 and BS2 are identical to the native xylanase of *B. subtilis*. Xylanase BS3 differs from the native enzyme by two amino acids and is resistant to the xylanase inhibitor present in flour. Xylanases BS2 and BS3 are used in baking applications to increase tolerance towards variations in process parameters, improve the dough, and increase the volume of baked goods. The xylanase preparation containing xylanase BS1 is not intended for commercialisation.

Summary of available safety information

JECFA evaluated xylanase from *Bacillus subtilis* in 2004 (WHO, 2004b), examining the methods used to construct the production strain, acute and short-term toxicity, as well as genotoxicity. No long-term studies were available.

The Committee allocated an ADI 'not specified' for xylanase from the genetically modified strain of *B. subtilis*, used in applications specified and in accordance with good manufacturing practice. The Committee noted that the xylanases would be denatured at temperatures >50˚C and would not be enzymatically active in food as consumed. Two specification monographs were prepared for xylanase preparations containing xylanase BS2 and BS3, the respective titles being *Xylanase from Bacillus subtilis expressed in B. subtilis*, and *Xylanase (resistant to xylanase inhibitor) from Bacillus subtilis containing a modified xylanase gene from B. subtilis*.

Both xylanase preparations conform to the *General specifications and considerations for enzyme preparations used in food processing* (FAO 2001). The Committee also noted that *B. subtilis* has been a source of enzymes used in food for many years.

Construction of the production strain

Three production strains for xylanases BS1, BS2 and BS3 were developed by transformation of the *B. subtilis* host strain with an appropriate vector. The host strain is derived from the well-characterised, non-pathogenic and non-toxigenic *B. subtilis* wild-type strain 168. Three transformation vectors were constructed, containing the xylanase gene from B. subtilis strain 168. Two vectors encode xylanases BS1 and BS2, both of which are identical to the wild-type xylanase. The vector encoding xylanase BS1 also contains the kanamycin resistance gene. The kanamycin resistance gene was removed from the vector encoding xylanase BS2. The vector encoding xylanase BS3 was genetically modified by two amino acid substitutions to make the encoded xylanase enzyme resistant to xylanase inhibitor present in flour. No antibiotic resistance markers are present on the vector encoding xylanase BS3. The introduced DNA is well characterised and would not result in the production of any toxic or undesirable substances. The production strains are stable with respect to the introduced DNA.

Acute and short term toxicity

Toxicological studies were done with different test batches of the three enzyme preparations, each being water-soluble, liquid concentrates from a fermentation with the respective production strain.

Acute toxicity studies with each of the three xylanase preparations were undertaken in rats. The LD_{50} in all cases was >2000 mg/kg bodyweight (equivalent to 200,000-220,000 total xylanase units(TXU)/kg bodyweight).

Groups of 5 male and 5 female rats were administered xylanase BS3 at a dose equivalent to 0, 20,000, 50,000 and 200,000 TXU/kg bodyweight/day by gavage for 4 weeks. No treatment related changes were observed in any of the parameters examined. The NOEL was 200,000 TXU/kg bodyweight/day (equivalent to an intake of TOS of 304 mg/kg bodyweight/day), the highest dose tested.

Groups of 10 male and 10 female rats were administered xylanase BS1 at a dose equivalent to 0, 8,000, 20,000 or 80,000 TXU/kg bodyweight/day by gavage for 13 weeks. No treatment related toxicologically significant effects were seen. The NOEL was 80,000 TXU/kg bodyweight/day (equivalent to 63 mg TOS/kg bodyweight/day), the highest dose tested.

Genotoxicity

Three xylanase enzyme preparations, containing xylanase BS1, BS2 or BS3, were negative for mutagenicity in assays in bacterial cells. Xylanase BS1 was also negative in an assay for chromosomal aberrations in human lymphocytes.

Evaluation and conclusion

Xylanases from *B. subtilis* are enzymes of low oral toxicity and the production strain is both non-pathogenic and non-toxigenic to humans. There are **no toxicological or other safety concerns** with the use of xylanase from *B. subtilis* as a processing aid.

2.7 Mixed β-glucanase and xylanase enzyme preparation, produced by a strain of *Humicola insolens*

Background

The mixed β-glucanase (EC 3.2.1.6) and xylanase (EC 3.2.1.8) preparation is produced by fed-batch, submerged, pure culture fermentation of a strain of *H. insolens* that is non-pathogenic and non-toxigenic. The enzyme preparation contains two main activities, β-glucanase and xylanase, and several secondary activities, including cellulase, hemicellulase, pentosanase and arabinase. The preparation is used in beer brewing to hydrolyse β-glucans, pentosans and other gums. This reduces the viscosity of the solution and thereby increases the filtration rate of both wort and beer and improves beer clarity. The enzyme preparation may also be used by the alcohol industry. The mixed enzyme preparation is standardized on the main activity, β-glucanase.

Summary of available safety information

Mixed β-glucanase and xylanase preparation, produced by *H. insolens* was evaluated by JECFA in 2003 (WHO, 2004d). The available data reviewed by the Committee included short-term toxicity and genotoxicity studies. No long-term toxicity studies were available.

The Committee allocated an ADI 'not specified' to mixed β-glucanase/xylanase from the production organism *H. insolens*, used in the applications specified and in accordance with good manufacturing practice. The Committee noted that the enzyme preparation is added during the mashing process of beer-making and the enzymes are denatured and inactivated during the subsequent wort-boiling stage. The beer filtration process is likely to remove the denatured enzymes along with other insoluble materials. The preparation may also be used in the spirits industry; again, in this case, no enzymes or other organic solids are expected to be carried over in the final product because ethanol is removed by distillation from the fermentation mash containing the enzyme preparation. The Committee concluded that no residual enzymes are expected to be present in food processed using the mixed β-glucanase/xylanase preparation. The Committee was not aware of any other food uses for this enzyme mixture in which the enzymes might persist in the final product.

Short-term toxicity

Groups of 10 male and 10 female rats received water containing the mixed enzyme preparation at dose levels of 0, 1, 3.3, or 10.2 g/kg bw/day by oral gavage for 13 weeks. No significant treatment-related effects were observed. In the absence of any treatment related effects, the NOEL was the highest dose tested, 10.2 g/kg bw/day.

Genotoxicity

In vitro genotoxicity studies were conducted on the mixed β-glucanase/xylanase preparation. Negative results were obtained for a mutagenicity assay in bacteria and in an assay for chromosomal aberrations in mammalian cells.

Evaluation and conclusion

Mixed β-glucanase and xylanase preparation produced by *H. insolens* is an enzyme of low oral toxicity. The production organism is non-pathogenic and non-toxigenic to humans and can be regarded as a safe source of β-glucanase and xylanase. No residues are expected to remain in the final food.

There are no toxicological or other safety concerns with the use of mixed β-glucanase and xylanase preparation produced by *H. insolens* as a processing aid.

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WHO (2004c) *Safety evaluation of certain food additives and contaminants. (alpha-amylase from Bacillus licheniformis containing a genetically engineered alpha-amylase gene from B. licheniformis)*. Food Additives Series. No. 52, WHO, Geneva. http://www.inchem.org/documents/jecta/jecmono/v52je02.htm.

WHO (2004d) *Safety evaluation of certain food additives and contaminants. (mixed xylanase, beta-glucanase enzyme preparation produced by a strain of Humicola insolens)*. Food Additives Series. No. 52, WHO, Geneva. http://www.inchem.org/documents/jecta/jecmono/v52je07.htm.

Attachment 3

Review of nomenclature of currently permitted enzymes as processing aids and suggested amendments

Naming and classification of enzymes

Enzymes are principally classified and named according to the reaction they catalyse. The chemical reaction catalysed is the specific property that distinguishes one enzyme from another, and this is the basis for the classification and naming of enzymes. Each enzyme is assigned a recommended name; usually at the suggestion of the person who submits the details. The Nomenclature Committee of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB) allocates a four-digit EC number, the first three digits of which define the reaction catalysed and the fourth of which is a unique identifier (serial number). Each enzyme is also assigned a systematic name that uniquely defines the reaction catalysed. (IUBMB 1992)

Consideration of the nomenclature of enzymes currently permitted for use as processing aids

Each enzyme entry in the Code was compared to the Recommendations of the Nomenclature Committee of the IUBMB via their web-based listing of enzymes.

It was decided to keep the enzyme names as simple as practically possible, while retaining enough specificity to make the names meaningful. At the same time recognising that the EC number was the unique identifying feature of the enzyme listed. It was further noted that the EC number together with the approval of the source of the enzyme gave each entry a unique identity for approved use in food for Australia and New Zealand.

Citing Enzyme Numbers

The IUBMB state that when citing an enzyme number it should be preceded by EC and a space. Therefore it is recommended that there be a global editorial amendment to remove all the square brackets around the enzyme numbers in the Code.

Recommendation – Tables to clauses 15, 16 and 17
Delete all square brackets around the EC numbers.

This correctly cites the enzyme numbers as directed by the Nomenclature Committee of the IUBMB.

Detailed consideration of enzymes listed as permitted processing aids

PERMITTED ENZYMES OF ANIMAL ORIGIN

Enzyme	Source
Lipase EC [3.1.1.3]	Bovine stomach; salivary glands or forestomach of calf, kid or lamb; porcine or bovine pancreas
Pepsin EC [3.4.23.1]	Bovine or porcine stomach
Phospholipase A_2 EC [3.1.1.4]	Porcine pancreas
Thrombin EC [3.4.21.5]	Bovine or porcine blood
Trypsin EC [3.4.21.4]	Porcine or bovine pancreas

Comment on nomenclature of animal derived enzymes

Lipase, EC 3.1.1.3 - EC number OK
IUBMB common name: triacylglycerol lipase
IUBMB other names include: lipase

Pepsin, EC 3.4.23.1 - EC number OK
IUBMB recommended name: pepsin A
IUBMB other names include: pepsin

A minor layout error was noted in the Table to clause 15 where the letters 'EC' should be located on a new line immediately before the square bracket and not immediately after the name 'Pepsin' on the previous line.

Phospholipase A_2, EC 3.1.1.4 - EC number OK
IUBMB common name: phospholipase A_2

Thrombin, EC 3.4.21.5 - EC number OK
IUBMB common name: thrombin.

Trypsin, EC 3.4.21.4 – EC number OK
IUBMB common name: trypsin.

Regulatory Status

With the exception of thrombin, the enzymes listed as being derived from animals have been included in the Code since processing aids were first gazetted as Standard A16 (Australian *Food Standards Code*) on 4 April 1996. Thrombin was gazetted on 8 January 1999.

Recommendation – Table to clause 15
Amend the entry for Lipase to read 'Lipase, triacylglycerol'.

It was noted that there were a number of different 'lipase' entries in the Code. This change is also consistent with recent amendments to the Code for lipase, triacylglycerol sourced from microbial origin. This change retains some consistency with IUBMB enzyme nomenclature, by providing more accurate description of its function as a lipase.

This entry was considered together with all the other 'lipase' entries. We suggest this approach to listing in the Code as it provides a consistent approach with minimum changes to the Code.

Recommendation – Table to clause 15
Amend entry for Pepsin by reposition the letters 'EC' so that they appear on a new line with the enzyme number.

This corrects a minor presentation error.

PERMITTED ENZYMES OF PLANT ORIGIN

Enzyme	Source
β-Amylase EC [3.2.1.2]	Sweet potato (*Ipomoea batatas)*
Actinidin	Kiwifruit (*Actinidia deliciosa*)
Bromelain EC [3.4.22.4]	Pineapple stem (*Ananas comosus)*
Ficin EC [3.4.22.3]	*Ficus* spp.
Malt carbohydrases α–Amylase & ß-Amylase combined EC [3.2.1.1] / EC [3.2.1.2]	Malted cereals
Papain EC [3.4.22.2]	*Carica papaya*

Comment on nomenclature of plant derived enzymes

β-Amylase, EC 3.2.1.2 - EC number OK
IUBMB common name: β-amylase.

Actinidin, - the EC number is EC 3.4.22.14
IUBMB common name: actinidain
IUBMB other names include: actinidin.

Bromelain, EC 3.4.22.4 – Since 1992 the EC number of this enzyme has been transferred to EC 3.4.22.32 known as stem bromelain and EC 3.4.22.33 known as fruit bromelain. 'Bromelain' is one of several listed 'other names' for stem bromelain.

Ficin, EC 3.4.22.3 - EC number OK
IUBMB common name: ficain
IUBMB other names include: ficin.

Malt carbohydrases α–Amylase & ß-Amylase combined, EC 3.2.1.1 / EC 3.2.1.2 –
α–Amylase, EC 3.2.1.1: EC number OK
IUBMB common name: α–amylase
ß-Amylase, EC 3.2.1.2: EC number OK
IUBMB common name**:** β-amylase.
These enzymes are listed individually and no mention is made of the name 'malt carbohydrases'.

Papain, EC 3.4.22.2 - EC number OK
IUBMB common name: papain.

Regulatory Status
The enzymes listed as being derived from plants have been included in the Code since Standard A16 – Processing Aids was first gazetted on 4 April 1996.

Recommendation – Table to clause 16
Amend entry for Bromelain by replacing it with 'Bromelain, stem EC 3.4.22.32'.

Since the approved source of the enzyme was from pineapple stem (*Ananas comosus*) it was not considered appropriate to extend the enzyme approval to fruit bromelain, which is sourced primarily from pineapple fruit.

Comment was also received stating that 'bromelain is now commercially sourced from *Ananas bracteatis*', but it was agreed that this review is not a vehicle for new permissions for enzymes.

Further consideration
Following circulation to EAG, one industry comment suggested that pineapple fruit bromelain (*Ananus comosus*) should also be listed. This approach was not supported because it was viewed as extending the permission for bromelain and was recognised as being beyond the scope of this review. The company is free to lodge a formal application to amend this part of the Code.

Recommendation – Table to clause 16
Delete the full entry for 'malt carbohydrases…' and include individual entries for α-amylase and ß-amylase derived from malted cereals. This will result in a new entry for α-amylase from malted cereals and an additional plant source entry - 'malted cereals' for ß-amylase.
We supported the argument put forward by Enzymes Solutions that as it was permissible to use the two enzymes together, even though they are sourced separately, then it was sensible to provide for individual permissions.

Further consideration
Following circulation to EAG, Genencor suggested the permission of source for this enzyme is extended from malted cereals to ungerminated barley.

FSANZ did not support this suggestion as it was beyond the scope of the review. The Company can lodge an application to amend the code if they wish.

Recommendation - Table to clause 16
Insert 'EC 3.4.22.14' in association with the entry for Actinidin.

This corrects the omission of an enzyme number for actinidin.

PERMITTED ENZYMES OF MICROBIAL ORIGIN

Enzyme	Source
α–Acetolactate decarboxylase EC [4.1.1.5]	*Bacillus subtilis* *Bacillus subtilis*, containing the gene for α–Acetolactate decarboxylase isolated from *Bacillus brevis*

EC number OK
IUBMB common name: Acetolactate decarboxylase
IUBMB other names include: α–Acetolactate decarboxylase

This enzyme was incorporated into the Code when Standard A16 was gazetted on 4 April 1996.

Recommendation – No change

We agreed to retain this entry, as it is a widely used term that is consistent with the current bounds of IUBMB Enzyme Nomenclature.

Aminopeptidase EC [3.4.11.1]	*Lactococcus lactis* *Aspergillus oryzae*

EC number OK
IUBMB common name: leucyl aminopeptidase
IUBMB other names include: leucine aminopeptidase and aminopeptidase I.

The nomenclature is not consistent with IUBMB. Aminopeptidase is the name of all enzymes in the 3.4.11.x reaction category of which there are 20 different amino peptidases listed. The enzyme EC 3.4.11.1 is commonly referred to as 'leucyl aminopeptidase'.

This enzyme was incorporated into the Code when Standard A16 was gazetted on 4 April 1996.

Recommendation – Table to clause 17

Amend the entry for 'aminopeptidase' to read 'leucyl aminopeptidase' but seek comment from enzyme suppliers in Australia as to the implication of this recommendation.

We wish to be consistent with the IUBMB enzyme nomenclature while using a name that shows a reasonable amount of specificity in relation to the function of the enzyme.
Comment was sought from industry EAG members to confirm that leucyl aminopeptidase (from the source enzymes listed) is the actual enzyme being used and not any of the other type of aminopeptidase.

Further consideration
As no comment was received following EAG consultation we agreed to proceed with the suggested recommendation.

α–Amylase EC [3.2.1.1]	*Aspergillus niger* *Aspergillus oryzae* *Bacillus licheniformis* *Bacillus licheniformis*, containing the gene for α-Amylase isolated from *Bacillus s stearothermophilus* *Bacillus subtilis* *Bacillus subtilis*, containing the gene for α-Amylase isolated from *Bacillus stearothermophilus*

EC number OK
IUBMB common name: α-Amylase

Industry suggested changing *Bacillus stearothermophilus* to *Geobacillus stearothermophilus* to reflect more recent name changes of the micro-organism.

This enzyme was incorporated into the Code when Standard A16 was gazetted on 4 April 1996.

Recommendation - Table to clause 17

For the entry of α-amylase amend occurrences of 'Bacillus stearothermophilus' to read 'Geobacillus stearothermophilus'. Consequentially include a statement in the editorial note reflecting this change in nomenclature of bacteria.

The suggestion from an industry stakeholder to insert this statement is supported, as this comment is correct.

β-Amylase EC [3.2.1.2]	*Bacillus subtilis*

EC number OK
IUBMB common name: β-Amylase

This enzyme was incorporated into the Code when Standard A16 was gazetted on 4 April 1996.

Recommendation
No change.

Arabinase EC [3.2.1.99]	*Aspergillus niger*

EC number OK
IUBMB common name: arabinan endo-1,5- α-L-arabinosidase
IUBMB other names include: endo-arabanase.

This enzyme was incorporated into the Code when Standard A16 was gazetted on 4 April 1996.

Recommendation - Table to clause 17
Amend entry for Arabinase to read 'Endo-arabanase'.

We agreed to use the term 'endo-arabanase, as it is a widely used term that is consistent with the current bounds of IUBMB Enzyme Nomenclature.

Arabino-furanosidase EC [3.2.1.55]	*Aspergillus niger*

EC number OK
IUBMB common name: α-*N*-arabinofuranosidase
IUBMB other names include: arabinosidase; α-arabinosidase; α-arabinofuranosidase.

This enzyme was incorporated into the Code between January 2000 and 20 December 2000.

Recommendation - Table to clause 17
Amend the entry for Arabino-furanosidase to read 'α-Arabinofuranosidase'.

We support a minor change to the entry because it is commonly used in literature while still being consistent with IUBMB enzyme nomenclature.

Carboxyl proteinase EC [3.4.23.6]	*Aspergillus melleus* *Aspergillus niger* *Aspergillus oryzae* *Rhizomucor miehei*

IUBMB entry for carboxyl proteinase EC 3.4.23.6 was transferred to the following list of 12 enzymes and was subsequently deleted in 1992.

EC 3.4.23.18 aspergillopepsin I;
EC 3.4.23.19 aspergillopepsin II;
EC 3.4.23.20 penicillopepsin;
EC 3.4.23.21 rhizopuspepsin
EC 3.4.23.22 endothiapepsin
EC 3.4.23.23 mucorpepsin
EC 3.4.23.24 candidapepsin
EC 3.4.23.25 saccharopepsin
EC 3.4.23.26 rhodotorulapepsin
EC 3.4.23.27 physaropepsin
EC 3.4.23.28 acrocylindropepsin
EC 3.4.23.30 pycnoporopepsin

The current Code entry for this enzyme was incorporated into the Code when Standard A16 was gazetted on 4 April 1996 (Amendment 29).

Recommendation - Table to clause 17
Suggest the deletion of carboxyl proteinase, with the possibility of inserting those updated enzymes where industry can provide adequate documentation that they are from the already approved source of micro-organisms.

Information was sought from industry on which enzyme (and its source) does industry use. It was agreed that official technical documentation in support of this advice should also be provided as proof of the source of the enzyme.

Further consideration
Industry members of the External Advisory Group advised that:
aspergillopepsin I (EC 3.4.23.18) derived from *Aspergillus niger* or *Aspergillus oryzae;*
aspergillopepsin II (EC 3.4.23.19) derived from *Aspergillus niger* ; and
mucorpepsin (EC 3.4.23.23) derived from *Rhizomucor miehei* were suggested replacements for carboxyl proteinase.

It is agreed to base the proposed changes to the Code for carboxyl proteinase on this industry response. This change will also be dependent on industry being able to provide official documentation confirming the specific microbial source(s) of the three enzymes mentioned.

Catalase EC [1.11.1.6]	*Aspergillus niger* *Micrococcus luteus*

EC number OK
IUBMB common name: catalase.
This enzyme was incorporated into the Code when Standard A16 was gazetted on 4 April 1996 (Amendment 29).

Recommendation
No change.

Cellulase EC [3.2.1.4]	*Aspergillus niger* *Trichoderma reesei* *Trichoderma viride*

EC number OK
IUBMB common name: cellulase.

This enzyme was incorporated into the Code when Standard A16 was gazetted on 4 April 1996 (Amendment 29).

Recommendation
No change.

Chymosin EC [3.4.23.4]	*Aspergillus niger* *Escherichia coli* K-12 strain GE81 *Kluyveromyces lactis*

EC number OK
IUBMB common name: chymosin

Recommendation
No change.

Cyclodextrin glucanotransferase EC [2.4.1.19]	*Paenibacillus macerans*

EC number OK
IUBMB common name: cyclomaltodextrin glucanotransferase
IUBMB other names include: cyclodextrin glucanotransferase.

This enzyme was incorporated into the Code between January 2000 and 20 December 2000.

Recommendation
No change.

We support the simplest entry taking into consideration the consistency with IUBMB enzyme nomenclature and accuracy of the name with respect to the function of the enzyme.

Dextranase EC [3.2.1.11]	*Chaetomium gracile* *Penicillium lilacinum*

EC number OK
IUBMB common name: dextranase
This enzyme was incorporated into the Code when Standard A16 was gazetted on 4 April 1996.

Recommendation
No change.

Esterase EC [3.1.1.1]	*Rhizomucor miehei*

EC number OK
IUBMB common name: carboxylesterase
IUBMB other names include: 25 ‘other names’ listed but none are ‘esterase’.
IUBMB categorization indicates that all the group of hydrolases, 3.1.x.x, act on ester bonds.
All other names appear to be more specific than ‘esterase’.

This enzyme was incorporated into the Code when Standard A16 was gazetted on 4 April 1996.

Recommendation
Replace the entry for ‘Esterase’ with ‘Carboxylesterase’.

Although the recommended replacement entry is more complex than ‘esterase’ it does reflect more accurately the function of the enzyme. It is also consistent with the IUBMB enzyme nomenclature.

α-Galactosidase EC [3.2.1.22]	*Aspergillus niger*

EC number OK
IUBMB common name: α-galactosidase
This enzyme was incorporated into the Code when Standard A16 was gazetted on 4 April 1996.

Recommendation
No change.

β-Glucanase EC [3.2.1.6]	*Aspergillus niger* *Aspergillus oryzae* *Bacillus subtilis* *Disporotrichum dimorphosporum* *Humicola insolens* *Talaromyces emersonii* *Trichoderma reesei*

EC number OK
IUBMB common name: endo-1,3(4)- β-glucanase
IUBMB other names include: There are 13 ‘other names’ although similar to β-Glucanase, they are more specific, for example endo-β-1,3-glucanase; endo-1,3-1,4- β-D-glucanase; or endo-1,3-β-D-glucanase.

This enzyme was incorporated into the Code when Standard A16 was gazetted on 4 April 1996.

Recommendation - No change.
We supported retaining the current entry as it retains specificity while retaining simplicity, noting it is not consistent with IUBMB enzyme nomenclature.

Glucoamylase EC [3.2.1.3]	*Aspergillus niger* *Aspergillus oryzae* *Rhizopus delemar* *Rhizopus oryzae* *Rhizopus niveus*

EC number OK
IUBMB common name: glucan 1,4-α-glucosidase
IUBMB other names include: glucoamylase.

This enzyme was incorporated into the Code when Standard A16 was gazetted on 4 April 1996.

Recommendation
No change.

Glucose isomerase or glucose isomerase xylose isomerase EC [5.3.1.5]	*Actinoplanes missouriensis* *Bacillus coagulans* *Microbacterium arborescens* *Streptomyces olivaceus* *Streptomyces olivochromogenes* *Streptomyces murinus* *Streptomyces rubiginosus*

EC number OK
IUBMB common name: xylose isomerase
IUBMB other names include: D-xylose isomerase.

Of the entry in the Code only the name 'xylose isomerase' is consistent with the current IUBMB enzyme nomenclature.

This enzyme was incorporated into the Code when Standard A16 was gazetted on 4 April 1996. Two additional microbial sources where added to the list before October 2002, i.e. *Streptomyces olivaceus,* and *Streptomyces olivochromogenes.*

Recommendation - Table to clause 17
Amend entry for glucose isomerase by deleting the words 'Glucose isomerase or glucose isomerase xylose isomerase' and replacing them with 'xylose isomerase'.

We support the simplest entry taking into consideration the consistency with IUBMB enzyme nomenclature and accuracy of the name with respect to the function of the enzyme.

Glucose oxidase EC [1.1.3.4]	*Aspergillus niger* *Aspergillus oryzae,* containing the gene for glucose oxidase isolated from *Aspergillus niger*

EC number OK
IUBMB common name: glucose oxidase.

This enzyme was incorporated into the Code when Standard A16 was gazetted on 4 April 1996. Subsequently approval of *Aspergillus oryzae,* containing the gene for glucose oxidase isolated from *Aspergillus niger* was gazetted on 27 Feb 2003.

Recommendation
No change.

α-Glucosidase (maltase) EC [3.2.1.20]	*Aspergillus oryzae* *Aspergillus niger*

EC number OK
IUBMB common name: α-glucosidase
IUBMB other names include: maltase

This enzyme was incorporated into the Code between January 2000 and 20 December 2000.

Recommendation - Table to clause 17
For the entry α-Glucosidase, delete '(maltase)'.

This removes additional names, which in turn simplifies the entry while remaining consistent with IUBMB Enzyme nomenclature.

β-Glucosidase EC [3.2.1.21]	*Aspergillus niger*

EC number OK
IUBMB common name: β-glucosidase
This enzyme was incorporated into the Code as part of the gazettal of Standard A16 – Processing aids on 4 April 1996.

Recommendation
No change.

β-Glucosidase exo-1,3 EC [3.2.1.58]	*Trichoderma harzianum*

EC number OK
IUBMB common name: glucan 1,3-β-glucosidase
IUBMB other names include: exo-1,3-β-glucosidase

This enzyme was incorporated into the Code between January 2000 and 20 December 2000.

Recommendation - Table to clause 17
Remove 'β-Glucosidase exo-1,3' and replace it with 'glucan 1,3-β-glucosidase'.

Because the Code entry is not strictly correct we preferred to see the IUBMB listed 'common name' which is still relatively simple, but was a more accurate description of the function of the enzyme.

Hemicellulase endo-1,3-β-xylanase EC [3.2.1.32]	*Humicola insolens*

EC number OK
IUBMB common name: xylan endo-1,3- β-xylosidase
IUBMB other names include: endo-1,3-β-xylanase.

This enzyme was incorporated into the Code when Standard A16 was gazetted on 4 April 1996.

Recommendation - Table to clause 17
Delete the word 'Hemicellulase' to leave the entry 'Endo-1,3-β-xylanase'.

This simplifies the entry by removing the outdated term 'hemicellulase' while remaining consistent with IUBMB Enzyme nomenclature.

Hemicellulase Endo-1,4- β-xylanase or xylanase EC [3.2.1.8]	*Aspergillus niger* *Aspergillus oryzae* *Aspergillus oryzae*, containing the gene for Hemicellulase endo-1,4-α-xylanase isolated from *Aspergillus aculeatus* *Aspergillus oryzae*, containing the gene for Hemicellulase endo-1,4-α-xylanase isolated from *Thermomyces lanuginosus* *Bacillus subtilis* *Humicola insolens* *Trichoderma reesei*

EC number OK
IUBMB common name: endo-1,4- β-xylanase
IUBMB other names include: β-D-xylanase.

This enzyme was incorporated into the Code when Standard A16 was gazetted on 4 April 1996. Permissions to use genetically modified *Aspergillus oryzae* were gazetted in December 1997 and May 1997 respectively.

Recommendation - Table to clause 17
(a) Delete the words 'Hemicellulase' and 'or xylanase' to leave the entry 'Endo-1,4-β-xylanase'.

(b) Amend the entry for permitted sources of micro-organism by deleting both occurrences of 'Hemicellulase' and replacing the Greek letter 'α' with the Greek letter 'β'. The latter corrects an editorial error.

This simplifies the entry by removing the outdated term 'hemicellulase' and 'xylanase' while remaining consistent with IUBMB Enzyme nomenclature. It also covers the consequential amendment associated with describing the approved source of the genetically modified enzyme.

A minor editorial error is corrected at the same time.

Hemicellulase multicomponent enzyme EC [3.2.1.78]	*Aspergillus niger* *Bacillus subtilis* *Trichoderma reesei*

EC number OK
IUBMB common name: mannan endo-1,4- β-mannosidase
IUBMB other names include: endo-1,4-β-mannanase or endo-β-mannanase.

This enzyme was incorporated into the code when Standard A16 was gazetted on 4 April 1996.

Recommendation - Table to clause 17
Replace 'Hemicellulase multicomponent enzyme' with 'Endo-1,4-β-mannanase'.

This removes a broad and outdated term i.e. 'hemicellulase multicomponent enzyme' and replaces it with a more accurate entry with respect to the IUBMB enzyme nomenclature.

Hexose oxidase EC [1.1.3.5]	*Hansenula polymorpha,* containing the gene for Hexose oxidase isolated from *Chondrus crispus*

EC number OK
IUBMB common name: hexose oxidase.

This enzyme was approved for use by means of Gazettal on 18 September 2003.

Recommendation
No change.

Inulinase EC [3.2.1.7]	*Aspergillus niger*

EC number OK
IUBMB common name: inulinase.

This enzyme was incorporated into the code when Standard A16 was gazetted on 4 April 1996.

Recommendation
No change.

Invertase EC [3.2.1.26]	*Saccharomyces cerevisiae*

EC number OK
IUBMB common name: β-fructofuranosidase
IUBMB other names include: invertase.

This enzyme was incorporated into the Code when Standard A16 was gazetted on 4 April 1996.

Recommendation
No change.

Staying with one of the listed 'other names' keeps the entry simple while remaining consistent with IUBMB enzyme nomenclature.

Lactase β-Galactosidase EC [3.2.1.23]	*Aspergillus niger* *Aspergillus oryzae* *Saccharomyces fragilis* *Saccharomyces lactis*

EC number OK
IUBMB common name: β-galactosidase
IUBMB other names include: lactase

This enzyme was incorporated into the Code when Standard A16 was gazetted on 4 April 1996.

Recommendation - Table to clause 17
Delete the word 'Lactase' and retain 'β-Galactosidase'.

This simplifies the entry while remaining consistent with IUBMB enzyme nomenclature but retaining a meaningful amount of specificity in its name.

Lipase, monoacylglycerol EC [3.1.1.23]	*Penicillium camembertii*

EC number OK
IUBMB common name: acylglycerol lipase
IUBMB other names include: monoacylglycerol lipase.

This enzyme was incorporated into the Code between January 2000 and 20 December 2000.

Recommendation - No change.

It was noted that 'lipase' is a generic term and that there are a number of different types of lipases permitted for use in the Code. This entry was considered together with all the other 'lipase' entries. We suggest this approach to listing in the Code as it provides a consistent approach with minimum changes to the Code.

Lipase, triacylglycerol EC [3.1.1.3]	*Aspergillus niger* *Aspergillus oryzae* *Aspergillus oryzae*, containing the gene for Lipase, triacylglycerol isolated from *Fusarium oxysporum* *Aspergillus oryzae*, containing the gene for Lipase, triacylglycerol isolated from *Humicola lanuginosa* *Aspergillus oryzae*, containing the gene for Lipase, triacylglycerol isolated from *Rhizomucor miehei* *Penicillium roquefortii* *Rhizopus arrhizus* *Rhizomucor miehei* *Rhizophus niveus* *Rhizophus oryzae*

EC number OK
IUBMB common name: triacylglycerol lipase
IUBMB other names include: lipase.

This enzyme was incorporated into the code when Standard A16 was gazetted on 4 April 1996. Permissions for the source organism were initially limited to *Aspergillus niger* and *Rhizomucor miehei*. The most recent permission includes *Penicillium roquefortii* as a source organism (3 August 06, amendment 87).

Recommendation
No change.

See comments for both lipase entries from both animal origin and microbial origin.

Lysophospholipase EC [3.1.1.5]	*Aspergillus niger*

EC number OK
IUBMB common name: lysophospholipase.

This enzyme was incorporated into the Code on 29 April 2004.

Recommendation
No change.

Maltogenic amylase EC [3.2.1.133]	*Bacillus subtilis* containing the gene for maltogenic amylase isolated from *Bacillus stearothermophilus*

EC number OK
IUBMB common name: glucan 1,4- α-maltohydrase
IUBMB other names include: maltogenic α-amylase

This enzyme was incorporated into the Code between 1 January 2000 and 20 December 2000.

Recommend - Table to clause 17
Amend the entry for maltogenic amylase by
(a) inserting 'α-' so that it reads 'Maltogenic α-amylase'; and
(b) replacing '*Bacillus stearothermophilus*' with '*Geobacillus stearothermophilus*'

We support the simplest entry taking into consideration the consistency with IUBMB enzyme nomenclature and accuracy of the name with respect to the function of the enzyme. Also amend the name of the source of the enzyme to reflect its current name.

Metalloproteinase EC [3.4.24.4]	*Aspergillus oryzae* *Bacillus subtilis* *Bacillus amyloliquefaciens* *Bacillus coagulans*

IUBMB entry for EC 3.4.24.4 was transferred to the following list of enzymes and this IUBMB entry was subsequently deleted in 1992.

EC 3.4.24.25 aeromonolysin,
EC 3.4.24.26 pseudolysin,
EC 3.4.24.27 thermolysin,
EC 3.4.24.28 bacillolysin,
EC 3.4.24.29 aureolysin,
EC 3.4.24.30 coccolysin,
EC 3.4.24.31 mycolysin,
EC 3.4.24.32 β-lytic metalloendopeptidase,
EC 3.4.24.39 deuterolysin,
EC 3.4.24.40 serralysin

Metalloproteinase was incorporated into the Code when Standard A16 was gazetted on 4 April 1996 (Amendment 29).

Recommendation - Table to clause 17
Deletion the entry for metalloproteinase#, with the possibility of inserting the following entries:
Bacillolysin produced from *Bacillus amyloliquefaciens;*
Bacillolysin produced from *Bacillus subtilis*;
Thermolysin produced from *Aspergillus oryzae*; and
Deuterolysin produced from *Aspergillus oryzae*

There is a proviso on this recommendation in that industry must be able to provide adequate documentation (such as technical data sheets) that specifies for each enzyme listed above the source micro-organism.

As this is no longer an internationally accepted name for this enzyme, we sought information from industry on which enzyme (and its source) does industry use. This information should be accompanied by official documentation in support of this advice.

Further consideration
EAG industry members have responded to the above request and have advised that metalloproteinase should be replaced with:
Bacillolysin produced from *Bacillus amyloliquefaciens;*
Bacillolysin produced from *Bacillus subtilis*;
Thermolysin produced from *Aspergillus oryzae*; and
Deuterolysin produced from *Aspergillus oryzae*

Mucorpepsin EC [3.4.23.23]	*Aspergillus oryzae* *Aspergillus oryzae*, containing the gene for Aspartic proteinase isolated from *Rhizomucor miehei* *Rhizomucor miehei* *Cryphonectria parasitica*

EC number OK
IUBMB common name: mucorpepsin.

This enzyme was incorporated into the Code between 1 January 2000 and 20 December 2000.

Recommendation - No change.

Further consideration
Comment received from Genencor about a typographic error, which has since been addressed. Genencor also made reference to a JECFA evaluation of this enzyme, but it was agreed that it was again beyond the scope of the review as the data was old.

Pectin lyase [EC 4.2.2.10]	*Aspergillus niger*

EC number OK
IUBMB common name: pectin lyase

This enzyme was incorporated into the Code between 1 January 2000 and 20 December 2000.

Recommend
No change.

Pectin methylesterase or Pectinesterase EC[3.1.1.11]	*Aspergillus niger* *Aspergillus oryzae,* containing the gene for pectinesterase isolated from *Aspergillus aculeatus*

EC number OK
IUBMB common name: pectinesterase.
IUBMB other names include: pectin methylesterase.

This enzyme was incorporated into the Code between January 2000 and 20 December 2000. An additional microbial source was added to the Code on 20 December 2001 (Amendment 58).

Recommendation - Table to clause 17
Amend the entry for 'Pectin methylesterase or Pectinesterase' by deleting 'Pectin methylesterase' and retain 'Pectinesterase'.

This simplifies the entry while remaining consistent with IUBMB Enzyme nomenclature.

Phospholipase A_1 EC [3.1.1.32]	*Aspergillus oryzae,* containing the gene for phospholipase A_1 isolated from *Fusarium venenatum*

EC number OK
IUBMB common name: phospholipase A_1.

This enzyme was incorporated into the Code on 3 August 2006 (Amendment 87).

Recommendation
No change.

Phospholipase A_2 EC [3.1.1.4]	*Streptomyces violaceoruber*

EC number OK
IUBMB common name: phospholipase A_2.

This enzyme was incorporated into the Code on 16 December 2004.

Recommendation
No change.

3-Phytase EC [3.1.3.8]	*Aspergillus niger*

EC number OK
IUBMB common name: 3-phytase.

This enzyme was incorporated into the Code on 4 April 1996 as phytase and was subsequently changed to 3-phytase at amendment 58 (20 December 2001).

Recommendation
No change.

6-Phytase EC [3.1.3.26]	*Aspergillus oryzae*, containing the gene for 6-phytase isolated from *Peniophora lycii*

EC number OK
IUBMB common name: 4-phytase
IUBMB other names include: 6-phytase (depending on which numbering system is used)

This enzyme was incorporated into the Code on 20 December 2001 (Amendment 58).

Recommendation - Table to clause 17
Amend entry for '6-phytase' by replacing the digit '6' with '4' for both occurrences of '6-phytase'.
Seek industry-specific comment on the implication of this amendment to the Code.

Polygalacturonase or Pectinase multicomponent enzyme EC [3.2.1.15]	*Aspergillus niger* *Aspergillus oryzae* *Trichoderma reesei*

EC number OK
IUBMB common name: polygalacturonase
IUBMB other names include: pectinase.

This enzyme was incorporated into the Code on 4 April 1996 (amendment 29) as pectinase multicomponent enzyme.

Recommendation - Table to clause 17
Amend the entry for 'Polygalacturonase or Pectinase multicomponent enzyme' by deleting the words 'or Pectinase multicomponent enzyme'.

This simplifies the entry while remaining consistent with IUBMB Enzyme nomenclature.

Pullulanase EC [3.2.1.41]	*Bacillus acidopullulyticus* *Bacillus licheniformis* *Bacillus subtilis* *Klebsiella pneumoniae*

EC number OK
IUBMB common name: pullulanase.

This enzyme was incorporated into the Code on 4 April 1996 (Amendment 29).

Recommendation
No change.

Serine proteinase EC [3.4.21.14]	*Bacillus lentus* *Bacillus licheniformis* *Bacillus subtilis* *Aspergillus oryzae*

IUBMB entry for EC 3.4.21.14 was transferred to the following list of enzymes and this IUBMB entry was subsequently deleted in 1992.

EC 3.4.21.62 subtilisin;
EC 3.4.21.63 oryzin;
EC 3.4.21.64 endopeptidase K;
EC 3.4.21.65 thermomycolin; and
EC 3.4.21.66 endopeptidase So

This enzyme was incorporated into the Code when Standard A16 was gazetted on 4 April 1996.

Recommendation - Table to clause 17
Suggest the deletion the entry for serine proteinase and insert an entry for
#Subtilisin EC 3.4.21.62 *Bacillus halodurans***
*Bacillus licheniformis**

Changes to this entry is on the proviso that industry can provide technical documentation that subtilisin is derived from the already approved source micro-organisms.

As this is no longer an internationally accepted name for this enzyme, FSANZ sought preliminary information from industry on which enzyme (and its source) does industry use. This information needs to be accompanied by official documentation in support of this advice to justify acceptance of the proposed enzyme.
This information will be the basis of providing recent replacements for the entry for serine proteinase.

Further consideration
Further information was sort from EAG members who worked in the industry. One member advised that Danisco/Genencor only suggested subtilisin produced from *Bacillus licheniformis* replace the entry for serine proteinase. The second member advised that Novozymes also suggest subtilisin produced from *Bacillus lentus* which is now classified as *B. halodurans.*

* Technical justification still needs to be received from Danisco/Genencor.
** Explanatory documentation has been provided by Novozymes which sufficiently justifies changing the microbiological source to read *Bacillus halodurans* rather than *Bacillus lentus.*

Transglucosidase EC [2.4.1.24]	*Aspergillus niger*

EC number OK
IUBMB common name: 1,4-α-glucan 6-α-glucosyltransferase
IUBMB other names include: oligoglucan-branching glycosyltransferase; 1,4-α-D-glucan 6-α-D-glucosyltransferase; D-glucosyltransferase.

The EC number is consistent with the current IUBMB list. However the name listed in the Code is different to both the common name, and 'other names' mentioned.

This enzyme was incorporated into the Code on 22 May 2003 (Amendment 66).

Recommendation – No change, however seek comment from industry on whether the simplicity of the current entry is sufficiently accurate given that it is not consistent with the IUBMB nomenclature.

Further consideration
FSANZ sought specific comment from EAG on any concerns associated with the above recommendation. No comments were raised although Genencor supported a change to the IUBMB common name.

FSANZ agreed to retain the original approach as reflected in the recommendation.

Transglutaminase EC [2.3.2.13]	*Streptomyces mobaraense*

EC number OK
IUBMB common name: protein-glutamine γ-glutamyltransferase
IUBMB other names include: transglutaminase.

This enzyme was incorporated into the Code on 8 January 1999 (Amendment 42).

Recommendation
No change.

Urease EC [3.5.1.5]	*Lactobacillus fermentum*

EC number OK
IUBMB common name: urease
IUBMB other names include: not listed.

This enzyme was incorporated into the Code on 29 April 04 (Amendment 70).

Recommendation
No change.

Editorial note:

Bacillus subtilis covers the strain known under the name *Bacillus amyloliquefaciens*.
The *Aspergillus niger* group covers strains known under the names *Aspergillus aculeatus, A. awamori, A. ficuum, A. foetidus, A. japonicus, A. phoenicis, A. saitor* and *A. usamii*.
Trichoderma reesei is also known as *Trichoderma longibrachiatum*.
Saccharomyces fragilis is also known as *Kluyveromyces fragilis* and *Kluyveromyces marxianus var. marxianus.*
Saccharomyces lactis is also known as *Kluyveromyces lactis*.
Mucor miehei is the former name for *Rhizomucor miehei*.
Micrococcus lysodeikticus is the former name for *Microccocus luteus*.
Bacillus macerans is the former name for *Paenibacillus macerans*.
Penicillium emersonii is the former name for *Talaromyces emersonii*.
Klebsiella aerogenes is the former name for *Klebsiella pneumoniae*.
Streptoverticillium mobaraense is the former name for *Streptomyces mobaraense*.
Humicola lanuginosa is also known as *Thermomyces lanuginosus*.
Mucor javanicus is also known as *Mucor circinelloides* f. *circinelloides*.
Penicillium roquefortii is also known as *Penicillium roqueforti*.

Analysis of the Editorial Note

Industry commented that *Bacillus amyloliquefaciens* is a separate species to *B. subtillus*. Since this has been confirmed it is suggested that the statement 'Bacillus subtilis covers the strain known under the name Bacillus amyloliquefaciens.' is no longer required in the editorial note. It was further noted that further information be sought from the enzyme industry to confirm which entries of Bacillus subtilis should actually read Bacillus amyloliquefaciens.

Recommendation – Editorial note
Delete the sentence '*Bacillus subtilis* covers the strain known under the name *Bacillus amyloliquefaciens*.'

Seek confirmation from industry about which enzymes sourced from *B. amyloliquefaciens* are they referring to as there are numerous entries for *B. subtilis*.

Comment from an industry stakeholder suggested that since 1967, *B. amyloliquefaciens* is described as a separate species, and should be treated as such. We have checked this comment and note that in fact it was only since 1987 that this is the case. FSANZ has sought advice from its EAG members and received advice on a number of entries that need amending.

Further consideration
The EAG industry members advised that the following enzyme entries be amended to be sourced from *B. amyloliquefaciens* and not from *B. Subtilis*:
α-amylase
β-amylase
endo-1,4-β-mannanase
endo-1,4- β-xylanase
bacillolysin
subtilisin

Recommendation – editorial note
Insert the sentence: '*Bacillus stearothermophilus* is the former name for *Geobacillus stearothermophilus.*'

Industry suggested changing *Bacillus stearothermophilus* to *Geobacillus stearothermophilus* to reflect more recent name changes of the micro-organism. This is consistent with current bacterial nomenclature.

Attachment 4

Summary of submissions

Round One

Company	Name
Genencor International Inc	Alice Caddow
Association of Manufacturers and Formulators of Enzyme Products (AMFEP)	Karolien De Neve
Australian Food and Grocery Council	Tony Downer
Enzyme Solutions Pty Ltd	Geoff Bearzatto
Food Technology Association Victoria	David Gill
Department of Agriculture, Fisheries and Forestry	Trent Brady
New Zealand Food Safety Authority	Carole Inkster
AMFEP	Huub Scheres
Queensland Health	Gary Bielby
F&N	Haydn Vesty

Submitter	Issues, comments
Association of Manufacturers and Formulators of Enzyme Products (AMFEP)	• The criteria in section 5.4 of the IAR is too narrow to identify which enzymes need to be reviewed. • Supports option 1 – maintain the status quo. • Enzymes which have been evaluated and approved by other internationals agencies according to international standards such as the European Scientific Committee for Food guidelines or equivalent should be exempted from the planned safety review. 1. Suggests that food enzymes have been shown from history to be inherently safe and there is no need to evaluate enzymes not examined since 1996, except those that have been identified by FSANZ or other appropriate parties to have a toxicological concern. 2. Suggests there is no good reason to delete any enzymes in the Code that may not be commercially used since will be very hard to know they are not being used (or may not be used in the future). 3. Supports the current situation where enzymes do not need to be labelled on food, and enzymes derived from GM sources where there is no novel DNA and/or protein in the final food. 4. Suggests only where a new food use of a current enzyme or the food use of a new enzyme is proposed should the by-products of enzyme reactions be considered. For other situations there is a history of safe use. 5. Suggests using the current Enzyme Commission of the International Union of Biochemistry nomenclature be used for the Standard. Also it suggests using current scientific references to classify and list production organisms. Frequent name changes to enzyme source names may cause confusion. Including former names is useful, though this information is available from scientific literature. Also name changes should not change the safety assessment of the enzyme or source. 6. Supports using the international harmonisation of evaluating enzymes approvals. It supports the JECFA specifications updated in 2001 for this purpose (FSANZ follows these and reference them in the Code, Std 1.3.4).

Submitter	Issues, comments
Genencor	Supports option 1, maintain the status quo of the standard, but with a proviso that a system be implemented to ensure consistency in enzymes and source organisms (point 5 above using international reference sources). The other parts of the submission, with the numbered points 1-6 and references are the same as that also received from AMFEP listed above.
Queensland Health	Supports option 2 – amend clauses 15, 16 and 17 of Standard 1.3.3 to update. The guidelines referred to in section 5.6 'must' (as opposed to 'should') consider the safety studies necessary to support permissions. They also favour specific attention for enzymes derived from genetically modified sources and the right of consumers to be made aware when enzymes from a genetically modified source is used in the production of the food.
Enzyme Solutions	Comments on the specific clauses. **Table to clause 15** •No comments **Table to clause 16** •Bromelain. Also sourced from *Ananas bracteatis*. The EC classification may have been expanded to include [3.4.22.32] and [3.4.22.33] (currently listed as EC [3.4.22.4] sourced from pineapple stem (Ananas comosus). •'Malt carbohydrases α-Amylase & β-Amylase combined'. Suggest should have the flexibility to be able to use the individual components, since it may be possible to produce either enzyme without the other being present. So replace current entry to 'α-amylase &/or β-amylase'. **Table to clause 17** •'Hemicellulase multicomponent enzyme' is too broad and open to abuse. Also suggests hemicellulase is old terminology and could be replaced by the more current names 'xylanase' or 'pentosanase' with appropriate EC numbers. •'Inulinase' EC [3.2.1.7] might also be referred to as β -mannanase. •'Metalloproteinase' EC [3.4.24.4]. AMFEP states there is no general IUB number for proteases, but general classification [3.4.2x.xx]. Often fungal sources are seen as EC [3.4.24.25/32/39/40] and bacterial sources as EC [3.4.24.28] from *Bacillus subtilis*. •'Serine proteinase' EC [3.4.21.14]. Often stated as being from [3.4.21.62/65/67] or as above stated with a general proteinase number (i.e. [3.4.2x.xx?]). •'Polygalacturonase or Pectinase multicomponent enzyme' EC [3.2.1.15]. Believed to be misleading, in that it suggests that the multicomponent pectinases are derived from the three listed organisms when most often (always?) the multicomponent is pectinases and cellulases blended together. The cellulases component will be sourced from Trichoderma reesei, which is listed in the table separately. They suggest the 'multicomponent' should not refer to cellulases but to pectinases which may be different forms. **Summary comments** Supports inclusion of enzymes that AMFEP have previously classified as safe, and similar nomenclature as far as possible. Regarding enzymes that are not currently used, would require surveying enzyme suppliers and the food industry.

Submitter	Issues, comments
Department of Agriculture, Fisheries and Forestry	Supports that any proposed amendments to the Standard that may come out of the review will be routine amendments that will not have any impact under the *Imported Food Control Act 1992*.
New Zealand Food Safety Authority	Supports option 2, to amend the clauses to update the current permissions for enzymes. NZFSA referred to an earlier New Zealand Government agencies' submission (to P188) on the draft Joint Code, dated 17 May 2000. This raised a number of concerns with the then new Standard 1.3.3 – Processing Aids. This Standard was based on A16 of the Australian *Food Standards Code* where chemicals were evaluated as part of the Proposal P86. However, extra processing aids have been included in Standard 1.3.3, which have not undergone such an evaluation. (This is now being performed by the new Proposals, P276 and P277). It therefore supports the safety assessment of enzymes, enzyme sources and by-products of enzymatic reactions. It also agrees with the proposed criteria in section 5.4 of the IAR, to evaluate the safety of the currently approved enzymes.
Australian Food and Grocery Council	AFGC states that the last review of processing aids in 1999 (P188) Final Assessment Report, formerly called Inquiry Report), made a statement, which is now subclause 17(2) of the Standard: 'The sources listed in the Table to this clause may contain additional copies of genes from the same organism'. It suggests because of this statement that FSANZ should consider a generic approval for any source microbial organism to contain inserted genes from any other already approved source microbial organism. It cautions against deleting the use of any enzymes not currently used in Australia or New Zealand, to include not currently used in 'any country' to ensure any deletions would not inhibit international trade.
Food Technology Association of Victoria	Supports option 2, to amend the Standard to update current permissions for enzymes, and to re-evaluate the safety of enzymes if there are any safety concerns to ensure section 10 objectives are met. Enzymes that have already been accepted by AMFEP that are also in the Code from the earlier industry survey of used enzymes should be accepted by FSANZ and not require further consideration. This would help ensure international consistency for food industries. Inquiries would be needed to suppliers and end users to ensure that enzymes that are not currently used may not be required in the future since if approvals are removed for such enzymes it will be costly and take time to seek re-approval. So only enzymes that all stakeholders agree are obsolete should be removed. All non-obsolete enzymes that have a long history of safe use should be retained in the Standard.

Submitter	Issues, comments
Food and Nutrition Australia	Enzymes sourced from GM organisms should be labelled on processed food produced using them, since currently enzymes, as processing aids, do not need to be labelled. As producers of emulsifiers, specifically for baked goods, suggested they have lost market share where manufacturers have replaced their emulsifiers (if acting as food additives requires labelling) with enzymes that do not need to be labelled. In summary, submits that enzymes are reclassified as ingredients requiring labelling or, that their GM status be declared on the label to enable consumers to make an informed choice. Either of these options will create a level playing field in our market.

Attachment 5

Terms of reference for the External Advisory Group

Within the scope of Proposal P276 – Review of Enzymes, the terms of reference for the External Advisory Group are to:

1. provide input and expert advice on amendments suggested to update clauses 15, 16 and 17 of Standard 1.3.3 relating to lists of approved enzymes and sources.

2. provide technical advice specifically in relation to:

- clause 15 - permitted enzymes of animal origin;
- clause 16 - permitted enzymes of plant origin; and
- clause 17 - permitted enzymes of microbial origin.

3. provide advice on:

- errors and anomalies within clauses 15, 16 and 17 of Standard 1.3.3 – Processing Aids;
- safety concerns with currently approved enzymes in the Code;
- enzymes currently not used by industry, however listed in the Code;
- safety concerns of by-products of enzymatic reactions; and
- consistency in the nomenclature of enzymes and the source organisms.