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Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

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FINAL ASSESSMENT REPORT

PROPOSAL P276

REVIEW OF PROCESSING AIDS (ENZYMES)

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

Executive Summary

Food Standards Australia New Zealand (FSANZ) has prepared Proposal P276 to review the regulation of enzymes as processing aids in clauses 15, 16 and 17 of Standard 1.3.3 – Processing Aids. A separate Proposal, P277 – Review of Processing Aids (other than Enzymes), was finalised and gazetted on 15 February 2007.

Standard A16, in the former Australian *Food Standards Code*, was formed as a result of Proposal P86 – Development of a Standard to Regulate the Use of Processing Aids, which reviewed the toxicity of processing aids. This Standard was gazetted in the former Australian *Food Standards Code* in April 1996.

Standard 1.3.3 – Processing Aids was established as a result of Proposal P188 – Review of Standard A16 – Processing Aids and was gazetted as part of the *Australia New Zealand Food Standards Code* (the Code) on 20 December 2000. Standard 1.3.3 was largely based on Standard A16 of the former Australian *Food Standards Code* with relevant New Zealand permissions for processing aids from the New Zealand *Food Regulations 1984*. New Zealand permissions for processing aids were added without full evaluation or detailed consultation with food industries in New Zealand. The review of the processing aids standard was a high priority of the New Zealand Government at the time of the review of the two countries' food standards. This Proposal (along with P277) was created to fulfil the desire to comprehensively review processing aid permissions.

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 were different for enzymes as distinct from other processing aids. Therefore matters considered as part of this review include:

- the safety of currently approved enzymes and by-products of enzymatic reactions and the guidelines for the safety assessment of enzymes;
- the 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 has not been 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.

Purpose

FSANZ has prepared Proposal P276 to review clauses 15, 16 and 17 of Standard 1.3.3 – Processing Aids, to specifically review the regulation of enzymes as processing aids.

Decision

FSANZ has reviewed clauses 15, 16 and 17 of Standard 1.3.3 – Processing Aids and has proposed a number of draft variations. These changes maintain public health and safety; and where practicable update nomenclature of enzymes and their sources; correct errors, remove duplications and anomalies, enhance consistency and improve the function of the Standard.

Reasons for Decision

Reasons for this decision are:

- The proposed amendments are consistent with the protection of public health and safety since no safety concerns were identified during the safety assessment.

- 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 regulatory alternatives that are more cost effective than the proposed amendments to Standard 1.3.3 – Processing Aids of the Code.

Consultation

The Initial Assessment Report for Proposal P276 was released for public comment from 17 December 2003 until 25 February 2004. Ten submissions were subsequently received. The Draft Assessment Report was released for public comment from 4 October 2006 to 29 November 2006. Eleven submissions were received during this period. A further two submissions were received after the closing date for public comment.

A summary of the submissions received during the first and second round of public comment appears at **Attachment 6**. FSANZ has taken the submitters' comments into account, in preparing the Final Assessment of this Proposal.

FSANZ established an External Advisory Group (EAG) drawn from experts in food enzymes and their regulation from the enzyme industry, regulatory agencies, academic and consumer groups. FSANZ staff held a meeting with the EAG in May 2004, and later communication seeking advice on the early proposed amendments was conducted via email.

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INTRODUCTION

1. Background

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 – Processing Aids from the former Australian *Food Standards Code.*

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

A separate Proposal, P277 – Review of Processing Aids (other than Enzymes), was finalised and gazetted on 15 February 2007 as part of the *Australia New Zealand Food Standards Code* – Amendment No. 91 – 2007. Proposal P277 provides a formal and comprehensive evaluation of permitted processing aids, other than enzymes, to formally harmonise the Australian and New Zealand regulations under the Code.

Proposal P276 focuses on the review of enzymes, separately from the other processing aids since FSANZ considers the safety assessment, risk management and technological issues are different for enzymes as distinct from other processing aids. The Initial Assessment Report for Proposal P276 was released for public comment from 17 December 2003 until 25 February 2004 while the Draft Assessment Report was released for public comment from 4 October 2006 to 29 November 2006.

1.1 Current Standard

The regulation of processing aids is covered by Standard 1.3.3 – Processing Aids of the Code. 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 a microbial source, the microbial source organism may contain additional copies of genes from the same organism.

1.2 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, processing aids were regulated in a mixed fashion. Some were listed by the National Health and Medical Research Council (NHMRC) in the *NHMRC Supplement to the Code* and others were individually approved and incorporated in a 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, 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).

1.3 Approval in Other Countries

The regulation of enzymes for Australia and New Zealand are contained within Standard 1.3.3, specifically, clauses 15, 16 and 17 of Standard 1.3.3 as described in section 1.1 above. That is, enzymes are considered processing aids.

The current system of regulation of processing aids in the Code for Australia and New Zealand differs from the regulation in many other countries. As processing aids, enzymes are regulated in the Code for Australia and New Zealand by being incorporated into a specific horizontal standard (meaning that the Standard applies generically across the whole of the food supply subject to specific provisions provided elsewhere in the Code).

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:

1.3.1 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. Enzyme preparations derived from genetically modified organisms are assessed for safety using the same scheme as non-GMO derived organisms.

1.3.2 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. Enzymes are listed by name only (i.e. no Enzyme Commission (EC) numbers). These entries also list specific sources, allowed applications and limits of use[1].

1.3.3 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.

1.3.4 USA

The following information relating to how enzymes are regulated in the USA has been taken from a submission to this Proposal[2].

In the USA, enzyme preparations (and processing aids) are regulated under the Federal Food, Drug and Cosmetic Act (FD&C Act). Enzymes may be classified as food additives or as Generally Recognized As Safe (GRAS) substances. Certain enzymes preparations are regulated as 'secondary' direct food additives under the Title 21 of the Code of Federal Regulations (CFR), Part 173 and may also be used in food if they are GRAS.

GRAS status may be achieved in three ways. First, substances may be affirmed as GRAS in 21 CFR Part 184 through a petition process (this process is no longer available). Second, a GRAS 'notification' may be submitted to the Federal Food and Drug Administration (FDA) under the interim policy for proposed changes to 21 CFR Part 170. Finally, the FD&C Act permits an individual or company to decide on its own ('self-affirm') that based on appropriate data, an ingredient is GRAS for a particular intended use, and to market the ingredient for that use without any prior contact with the agency.

[1] http://www.hc-sc.gc.ca/fn-an/alt_formats/hpfb-dgpsa/pdf/legislation/e_c-tables.pdf accessed 21 June 2007
[2] Submission from John Carroll, Chair of the Enzyme Technical Association (ETA), to this Proposal

1.3.5 European Union

In the European Union (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 European Commission (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 the former European Scientific Committee on Food (SCF) guidelines).
- Germany considers enzymes to be food additives but exempts them from approval (authorisation).
- The Netherlands does not have a specific enzyme regulation.
- 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.

On 27 July 2006, the European Commission published a proposal for an EU Regulation on food enzymes. According to this proposal, all food enzymes currently on the EU market will be evaluated for safety by the European Food Safety Authority (EFSA) and subsequently be placed on a positive list. As soon as the positive list has been published, enzymes not listed will require a pre-market approval[3]. It is not known how long this process will take.

2. The Issue

FSANZ has prepared Proposal P276 to specifically review the regulation of enzymes as processing aids in clauses 15, 16 and 17 of Standard 1.3.3. This Proposal was to run concurrently with Proposal P277 – Review of Processing Aids (other than Enzymes), which was finalised and gazetted on 15 February 2007.

This Proposal was created together with Proposal P277 to meet obligations to the New Zealand Government to comprehensively review processing aid permissions, following the review of the two countries' food standards and the creation of the joint *Australia New Zealand Food Standards Code* on 20 December 2000.

[3] Post Draft Assessment Report submission from Youri Skaskevitch, Association of Manufacturers and Formulators of Enzyme Products (AMFEP) Secretariat

3. Objectives

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

- the protection of public health and safety;
- 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 aim of this review is to protect public health and safety by ensuring enzymes are safe for use. A risk analysis has been undertaken using the best available scientific evidence. It is also important to promote consistency between domestic and international regulation of enzymes and to ensure the domestic food industry remains internationally competitive.

Matters being considered as part of this review include:

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

However, this review has not been 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.

4. Key Assessment Questions

In assessing this Proposal, FSANZ considered the following questions:

- Are the permitted enzymes safe?

- Is there a requirement to update the nomenclature for enzymes and their sources?

RISK ASSESSMENT

5. Safety Assessment Summary

5.1 Evaluation

Standard A16 in the former Australian *Food Standards Code* was formulated by Proposal P86 in 1996. However, 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[4] 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 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.

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*

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

[4] 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)

RISK MANAGEMENT

6. 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 as required.

7. Impact Analysis

7.1 Affected Parties

The affected parties to this Proposal are:

- food manufacturers who use enzymes in manufacturing and packaging their food products for sale in Australia and New Zealand which also includes the import of food into 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 Benefit Cost Analysis

This Proposal has reviewed specific clauses of Standard 1.3.3 in relation to the safety of currently approved enzymes, nomenclature used for enzymes and source organisms, and considered enzymes not currently used in Australia and New Zealand. However, it was not expected that there would be any major costs or impacts to food manufacturers, consumers or regulatory agencies.

7.2.1 Option 1 Status quo

7.2.1.1 Industry

Because this option does not change the Code there should not be any immediate impact on industry. It does however deny industry an improved level of clarification that would 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 the improved clarity of Standard 1.3.3 as being suggested in option 2.

7.2.2 Option 2 Amend clauses 15, 16, and 17 of Standard 1.3.3

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 associated with the review of enzyme permissions.

This Final Assessment has taken into account concerns raised by industry associated with unintended imposts on industry based on the draft variations as proposed at Draft Assessment. These have in the main been reconsidered and the associated draft variations to the Code have been changed.

Amending Standard 1.3.3 as a result of the review of enzymes does not impose any additional/discernable costs to the industry. This is reflected in the Business Cost Calculator Report, in accordance with the Office of Best Practice Regulation (OBPR) guidelines. This report is found at **Attachment 4**.

7.2.2.2 Consumers

The advantage for consumers is that the safety of a number of currently approved enzymes has been confirmed where more recent safety information was available. There is no other immediate effect on consumers of this option.

7.2.2.3 Government

Option 2 does not include approving new enzymes, therefore the impacts should be minimal for regulatory agencies. Inconsistencies associated with enzyme and source organism nomenclature have been minimised within the Code, thus reducing some unnecessary confusion and enquiries.

7.3 Comparison of Options

Option 2 is the preferred option. It has assessed and confirmed the safety of those enzymes where additional scientific data was available. In addition, amendments have been made to improve the clarity of the Standard, primarily based on recent international nomenclature changes for both enzymes and their source organisms.

COMMUNICATION AND CONSULTATION STRATEGY

8. Communication

FSANZ has applied a limited communication strategy to Proposal P276. This involved advertising the availability of assessment reports for public comment in the national press and making the reports available on the FSANZ website.

Individuals and organisations that made submissions on this Proposal were notified at each stage of the Proposal. If the FSANZ Board approves the Final Assessment Report, FSANZ will notify the Ministerial Council. If no review is sought by the Ministerial Council stakeholders, including the public, will be notified of the gazettal changes to the Code in the national press and on the website.

FSANZ provides an advisory service to the jurisdictions on changes to the Code.

9. Consultation

The Initial Assessment Report for Proposal P276 was released for public comment from 17 December 2003 until 25 February 2004. Ten submissions were subsequently received.

The Draft Assessment Report was released for public comment from 4 October 2006 to 29 November 2006. Eleven submissions were received during this period, 4 from Jurisdictions, 3 from enzyme producing companies, 3 from enzyme industry associations and 1 private individual. A further two submissions were received after the closing date for public comment. A summary of the submissions received during the first and second round of public comment appears at **Attachment 5**.

FSANZ has taken the submitters' comments into account in preparing the Final Assessment of this Proposal.

FSANZ established an External Advisory Group (EAG) to assist with this review. Members were drawn from experts with expertise in food enzymes and their regulation from industry groups, regulatory agencies, academic and consumer groups. FSANZ staff met with the EAG in May 2004. Expert advice was received on the early proposed amendments via email correspondence. Other later more specific advice was obtained from the enzyme industry.

In general there continues to be support in progressing the review of enzymes as processing aids, either expressed explicitly by five submitters or implicitly by the remaining submitters not raising any concerns.

Major issues raised and comments made in submissions are discussed and addressed below.

9.1 Removal of obsolete entries

No submitters identified entries from Standard 1.3.3 as being obsolete. Two of the four jurisdictional submitters specifically commented on retaining existing enzyme permissions where there is some question about whether or not they are obsolete. Concern was also raised that removal or alteration of current permissions may unintentionally create trade disruptions with resource implications to both jurisdictions and industry.

All enzyme companies and two industry-based associations supported retaining all current entries so as not to disrupt potential trade or resource concerns.

9.1.1 FSANZ consideration

As no enzymes have been identified as being obsolete, FSANZ is proposing that no enzymes be deleted.

9.2 Comment related to potential undesirable by-products

One submitter commented on a statement made in the safety assessment summary at Draft Assessment. The issue relates to the approval of enzymes and the possible creation of undesirable by-products due to the wide range of substrates that some enzymes may interact with. The submitter made the following statement to address this issue 'individual enzymes are very specific and do not act on a broad range of substrates. As a consequence, the reaction products are known and there is no need to question unintended by-products even if the enzyme is used in a broad range of foods containing the specific substrate the enzyme act upon.'

9.2.1 FSANZ consideration

This comment was noted. This section of the Draft Assessment Report has been removed from the Final Assessment.

9.3 Naming and classifying enzymes

FSANZ has undertaken a review of the nomenclature of currently permitted enzymes listed 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 changes to nomenclature appears in **Attachment 3**. Tables 2-4 include FSANZ's consideration of comments received following the public release of the Draft Assessment Report and its recommendations.

The following items provide a brief summary of the major issues raised by an evaluation of nomenclature.

9.3.1 Delete general entries where names have changed via IUBMB nomenclature

Comment was invited from the enzyme industry about the proposed deletion of the enzymes listed below and replacing them with enzymes nominated by industry but fitting into the IUBMB reclassification of each. Recommendations of the nomenclature committee of the IUBMB have transferred the following classification of enzyme into an expanded but more specific listing of enzymes. These more specific groups are based on their catalytic reactions.

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.

All industry submitters and industry-based associations recommended retaining the above four entries. They do not see any benefit in updating the names to align with revised IUBMB nomenclature, as it complicates the current positive enzyme permissions in Standard 1.3.3, rather than simplifies it. No product alternatives were provided by industry or their associations.

A comment in support of the industry position expressed above stated that 'not all existing enzyme proteins on the market are notified to the IUBMB and therefore lack a specific dedicated EC number, but fall within the existing general entries like proteinase or hemicellulase or cellulase.'

9.3.1.1 FSANZ consideration

FSANZ has reconsidered its recommendation to delete the above entries as it was dependant on industry providing appropriate alternatives. FSANZ accepts the industry recommendation to retain the above entries, noting that this will maintain a more simplified positive list of permitted enzymes for food use. It is also recognised that this recommendation, to retain the listing for these four entries, will provide industry with flexibility and product innovation associated with these entries. FSANZ also recognises that there is no change in the associated permitted source organisms and those approved are considered safe for this purpose.

In addition, jurisdictional comment included reluctance to delete entries where approval status of a product may have consequential trade disruptions and associated resource implications.

In considering the use of EC numbers, approval for enzymes will continue to be a pre-market process that is specific to a commercial enzyme preparation that is standardised to its major enzymatic activity. This assessment process is tied to a specific enzyme name and unique EC number as well as a defined source. If an enzyme, after a pre-market assessment is accepted then these details (name, EC number and source organism) will be used to list this specifically approved enzyme in the Code.

9.3.2 Aminopeptidase, Hemicellulases and Polygalacturonase

FSANZ had identified aminopeptidase, hemicellulases and polygalacturonase as several enzymes among many entries to be updated to align with revised IUBMB nomenclature of enzymes. Specific comments were received from the industry recommending the retention of these original entries.

9.3.2.1 FSANZ consideration

It was noted that no specific safety concerns were identified for these entries. It was also noted that the current entries gave a broad permission for use of these enzymes in foods and that amending these entries may unintentionally remove permissions of some current use.

FSANZ has therefore reconsidered its recommendation and accepts the industry advice to retain these enzyme names. **Attachment 3** provides an expanded discussion for each enzyme.

9.3.3 Updating nomenclature of source organisms

This review has also given FSANZ an opportunity to update the nomenclature of the source organisms. The need to retain editorial notes was considered and agreed as a positive means of clarifying these name changes. Detailed discussion associated with these changes also appears in **Attachment 3.**

It is recommended that the names of the following source organisms be changed and that appropriate amendments to the Editorial note also be made:

Bacillus stearothermophilus is the former name for *Geobacillus stearothermophilus;*
Saccharomyces lactis is the former name of *Kluyveromyces lactis*;
Saccharomyces fragilis and *Kluyveromyces fragilis* are both superseded by *Kluyveromyces marxianus*; and
Streptomyces mobaraense, is amended to reflect its correct name: *Streptomyces mobaraensis.*

9.3.4 Bacillus subtilis and Bacillus amyloliquefaciens

In the Draft Assessment Report FSANZ noted that since 1987 *Bacillus amyloliquefaciens* is no longer a strain of the species of *Bacillus subtilis*, but a species in its own right. FSANZ therefore sought additional comment from industry to confirm which of their enzymes are sourced from *Bacillus amyloliquefaciens* and which are sourced from *Bacillus subtilis*. FSANZ also recommended deleting the current related editorial statement without replacing it.

The Association of Manufacturers and Formulators of Enzyme Products (AMFEP), Danisco and the Enzyme Technical Association (ETA) recommend listing both the species, *Bacillus subtilis* and *Bacillus amyloliquefaciens,* for each entry where one or the other is the approved source organism. AMFEP provided the following detailed justification.

- Both species are able to produce the enzymes listed.
- Both species are safe and suitable hosts for the enzymes listed.
- Enzymes that were evaluated before 1987 were all classified as derived from *Bacillus subtilis.* It is not possible to determine in retrospect which of these would presently be classified as derived from *Bacillus amyloliquefaciens.*
- Present producers may not have in all cases re-classified the source organism.
- Most positive enzyme lists outside Australia/New Zealand, including JECFA, still list *Bacillus subtilis* based on the pre-1987 classification. Thus, in order to reflect current international standards, both organisms should be mentioned.

FSANZ consideration

FSANZ notes that the current entry in the editorial note effectively recognises *Bacillus amyloliquefaciens* as a permitted source organism wherever *Bacillus subtilis* is listed. Taking into account the above justifications and the current Code FSANZ has reconsidered its previous conclusions and recommends: With the exception of genetically modified enzymes, all entries that are sourced from *Bacillus subtilis* should also include a listing for *Bacillus amyloliquefaciens* as a permitted source organism.

FSANZ further agrees to include an appropriate editorial note to help explain the change in relationship of *Bacillus amyloliquefaciens* to *Bacillus subtilis*.

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 repositioning 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
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 α-acetolactate decarboxylase to insert an additional source: *Bacillus amyloliquefaciens*	This amendment recognises that *Bacillus amyloliquefaciens* is a separate species from *Bacillus subtilis* where currently our editorial note states '*Bacillus subtilis* covers the strain known under the name *Bacillus amyloliquefaciens'*.
Amend the entry of α-amylase so that (a) all occurrences of '*Bacillus stearothermophilus*' will now read '*Geobacillus stearothermophilus*'; and (b) insert an additional source: *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* where currently our editorial note states '*Bacillus subtilis* covers the strain known under the name *Bacillus amyloliquefaciens'*.
Amend the entry for β-amylase to insert an additional source: *Bacillus amyloliquefaciens*	This amendment recognises that *Bacillus amyloliquefaciens* is a separate species from *Bacillus subtilis* where currently our editorial note states '*Bacillus subtilis* covers the strain known under the name *Bacillus amyloliquefaciens'*.

Suggested action	Reason, comment
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.
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.
Amend the entry for β-glucanase to insert an additional source: *Bacillus amyloliquefaciens*	This amendment recognises that *Bacillus amyloliquefaciens* is a separate species from *Bacillus subtilis* where currently our editorial note states '*Bacillus subtilis* covers the strain known under the name *Bacillus amyloliquefaciens'*.
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-1,4- β-xylanase or xylanase' by (a) deleting the words 'or xylanase' are; (b) inserting an additional source: *Bacillus amyloliquefaciens;* and (c) replacing 'α' with 'β' in the source column.	These amendments will: (a) simplify the entry; (b) recognises *Bacillus amyloliquefaciens* as a separate species from *Bacillus subtilis* where currently our editorial note states '*Bacillus subtilis* covers the strain known under the name *Bacillus amyloliquefaciens'*; and (c) correct an editorial error.
Amend the entry for 'Hemicellulase multicomponent enzyme' by inserting an additional source: *Bacillus amyloliquefaciens.*	This amendment recognise *Bacillus amyloliquefaciens* as a separate species from *Bacillus subtilis* where currently our editorial note states '*Bacillus subtilis* covers the strain known under the name *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.

Suggested action	Reason, comment
Amend the entry for metalloproteinase by inserting an additional source: *Bacillus amyloliquefaciens.*	This amendment recognises *Bacillus amyloliquefaciens* as a separate species from *Bacillus subtilis* where currently our editorial note states '*Bacillus subtilis* covers the strain known under the name *Bacillus amyloliquefaciens'*.
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.
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 and is supported by industry.
Amend the entry for pullulanase by inserting an additional source: *Bacillus amyloliquefaciens.*	This amendment recognises *Bacillus amyloliquefaciens* as a separate species from *Bacillus subtilis* where currently our editorial note states '*Bacillus subtilis* covers the strain known under the name *Bacillus amyloliquefaciens'*.
Amend the entry for serine proteinase (a) by inserting an additional source: *Bacillus amyloliquefaciens;* and (b) by replacing the source: *Bacillus lentus* with *Bacillus halodurans.*	(a) This amendment recognises *Bacillus amyloliquefaciens* as a separate species from *Bacillus subtilis.* (b) *Bacillus halodurans* replaces *Bacillus lentus* for technical reasons.
Amend the entry for transglutaminase by replacing the word '*mobaraense*' with '*mobaraensis*'	This amendment corrects a source organism name.
Amend the **Editorial note** by (a) replacing the sentence: '*Bacillus subtilis* covers the strain known under the name *Bacillus amyloliquefaciens*.' with '*Bacillus amyloliquefaciens* as a separate species from *Bacillus subtilis'*. (b) inserting the sentence: '*Bacillus stearothermophilus* is the former name for *Geobacillus stearothermophilus.'*; (c) inserting the sentence: '*Saccharomyces fragilis* and *Kluyveromyces fragilis* are the former names for *Kluyveromyces marxianus.*'; (d) amend the last word in the sentence: '*Streptoverticillium mobaraense* is the former name for *Streptomyces mobaraense*' to read '*mobaraensis'*; and	(a) Since 1987, *B. amyloliquefaciens* is described as a separate species and therefore should be treated as such in the table to clause 17. (b) Stakeholder comment is supported that the entry for *Bacillus stearothermophilus* be updated to reflect its current name: *Geobacillus stearothermophilus.* (c) & (d) This updates the nomenclature of the microbiological source. (e) Addition of this sentence is a consequential amendment of renaming 6-phytase to read 4-phytase. (f) and (g) Inserting a new sentence picking up a former name, and amending a current note to reflect current nomenclature use.

Suggested action	Reason, comment
(e) inserting the sentence: '4-phytase is also known as 6-phytase.' (f) inserting the sentence saying '*Bacillus lentus* is the former name of *Bacillus halodurans.'* (g) amending the sentence '*Saccharomyces lactis* is also known as *Kluyveromyces lactis*' since *Saccharomyces lactis* is the former name, to '*Kluyveromyces lactis* – former name *Saccharomyces lactis'*.	

9.4 Other issues

One submitter to the Initial Assessment Report 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. Similarly a submitter to the Draft Assessment Report wanted the label on food produced using enzymes from a GM source to reflect the GM nature. They did not support approval of enzymes from GM sources as they believe they are neither proven to be safe nor supported by Australian consumers.

9.4.1 FSANZ consideration

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).

Enzymes, whether sourced from a GM source or not, are required to undergo a safety assessment to ensure they are safe for their proposed use, before they are approved. That is the case for all the enzymes approved in the Code. This review has reinforced the safety of the permitted enzymes.

9.5 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 no relevant international standards (CODEX) for enzymes and amending the *Australia New Zealand Food Standards Code* (the Code) to primarily update the enzyme names is unlikely to have a significant effect on international trade.

FSANZ notified the WTO of the proposed changes to the Code as a means of widening our consultation and specifically seeking international information on four enzymes (i.e. 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) that are no longer considered current by the International Union of Biochemistry and Molecular Biology. In particular FSANZ sought:

- comment on the appropriate replacement enzymes and their sources;
- information on possible trade implications on proposed amendments to the Code; and
- information about international usage of enzymes.

Notification of the proposed changes to the Code was made to the WTO in accordance with the WTO Technical Barriers to Trade Agreement (TBT). This enabled other WTO member countries to comment on proposed changes to standards where they may have a significant impact on them.

No comments were received from WTO member countries in response to these notifications.

CONCLUSION

10. Decision

Decision

FSANZ has reviewed clauses 15, 16 and 17 of Standard 1.3.3 – Processing Aids and has proposed a number of draft variations. These changes maintain public health and safety; and where practicable update nomenclature of enzymes and their sources; correct errors, remove duplications and anomalies, enhance consistency and improve the function of the standard.

10.1 Reasons for Decision

Reasons for this decision are:

- The proposed amendments are consistent with the protection of public health and safety based on no safety concerns being identified during the safety assessment.

- 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 Standard 1.3.3 – Processing Aids of the Code.

11. Implementation and Review

It is proposed that the draft variations come into effect on the date of gazettal.

ATTACHMENTS

1. Draft variations to the *Australia New Zealand Food Standards Code*
2. Safety Assessment Report, Toxicological, Nutritional, Food Technology, Dietary Assessment, Microbiology Reports)
3. Review of the nomenclature of currently permitted enzymes and their approved sources.
4. Business Cost Calculator Report: Proposal P276 – Review of Processing Aids (Enzymes)
5. Summary of issues raised in public submissions in the first and second rounds

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 –*

Table to clause 15

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 –*

Table to clause 16

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 3.4.22.14	Kiwifruit (*Actinidia deliciosa*)
Bromelain EC 3.4.22.4	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 –*

Table to clause 17

Enzyme	Source
α–Acetolactate decarboxylase EC 4.1.1.5	*Bacillus amyloliquefaciens* *Bacillus subtilis* *Bacillus subtilis*, containing the gene for α–Acetolactate decarboxylase isolated from *Bacillus brevis*
Aminopeptidase EC 3.4.11.1	*Aspergillus oryzae* *Lactococcus lactis*
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* *Bacillus subtilis*, containing the gene for α-Amylase isolated from *Geobacillus stearothermophilus* *Geobacillus stearothermophilus*
β-Amylase EC 3.2.1.2	*Bacillus amyloliquefaciens* *Bacillus subtilis*
α–Arabinofuranosidase EC 3.2.1.55	*Aspergillus niger*
Carboxyl proteinase EC 3.4.23.6	*Aspergillus melleus* *Aspergillus niger* *Aspergillus oryzae* *Rhizomucor miehei*
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*
Dextranase EC 3.2.1.11	*Chaetomium gracile* *Penicillium lilacinum*
Endo-arabinase EC 3.2.1.99	*Aspergillus niger*
α-Galactosidase EC 3.2.1.22	*Aspergillus niger*
β-Galactosidase EC 3.2.1.23	*Aspergillus niger* *Aspergillus oryzae* *Kluyveromyces marxianus* *Kluyveromyces lactis*
Glucan 1,3-β-glucosidase EC 3.2.1.58	*Trichoderma harzianum*

Enzyme	Source
β-Glucanase EC 3.2.1.6	*Aspergillus niger* *Aspergillus oryzae* *Bacillus amyloliquefaciens 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*
Hemicellulase endo-1,3-β-xylanase EC 3.2.1.32	*Humicola insolens*
Hemicellulase 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* *Bacillus subtilis* *Humicola insolens* *Trichoderma reesei*
Hemicellulase multicomponent enzyme EC 3.2.1.78	*Aspergillus niger* *Bacillus amyloliquefaciens* *Bacillus subtilis* *Trichoderma reesei*
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*
Lipase, monoacylglycerol EC 3.1.1.23	*Penicillium camembertii*

Enzyme	Source
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* *Hansenula polymorpha,* containing the gene for Lipase, triacylglycerol isolated from *Fusarium heterosporum* *Mucor javanicus* *Penicillium roquefortii* *Rhizopus arrhizus* *Rhizomucor miehei* *Rhizophus niveus* *Rhizophus oryzae*
Lysophospholipase EC 3.1.1.5	*Aspergillus niger*
Maltogenic α-amylase EC 3.2.1.133	*Bacillus subtilis* containing the gene for maltogenic α-amylase isolated from *Geobacillus stearothermophilus*
Metalloproteinase	*Aspergillus oryzae* *Bacillus amyloliquefaciens* *Bacillus coagulans* *Bacillus subtilis*
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 or Pectinase multicomponent enzyme EC 3.2.1.15	*Aspergillus niger* *Aspergillus oryzae* *Trichoderma reesei*
Pullulanase EC 3.2.1.41	*Bacillus acidopullulyticus* *Bacillus amyloliquefaciens* *Bacillus licheniformis* *Bacillus subtilis* *Klebsiella pneumoniae*
Serine proteinase EC 3.4.21.14	*Aspergillus oryzae* *Bacillus amyloliquifaciens* *Bacillus halodurans* *Bacillus licheniformis* *Bacillus subtilis*

Enzyme	Source
Transglucosidase EC 2.4.1.24	*Aspergillus niger*
Transglutaminase EC 2.3.2.13	*Streptomyces mobaraensis*
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 the* Editorial note*, immediately following the* Table to clause 17, *substituting –*

Editorial note:

Bacillus amyloliquefaciens is a separate species from *Bacillus subtilis*.
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 also known as *Trichoderma longibrachiatum*.
Kluyveromyces marxianus - former names *Saccharomyces fragilis* and *Kluyveromyces fragilis*.
Kluyveromyces lactis - former name *Saccharomyces lactis*.
Rhizomucor miehei - former name *Mucor miehei*.
Micrococcus luteus - former name *Micrococcus lysodeikticus*.
Paenibacillus macerans - former name *Bacillus macerans*.
Talaromyces emersonii - former name *Penicillium emersonii*.
Klebsiella pneumoniae - former name *Klebsiella aerogenes*.
Streptomyces mobaraensis - former name *Streptoverticillium mobaraensis*.
Humicola lanuginosa also known as *Thermomyces lanuginosus*.
Mucor javanicus also known as *Mucor circinelloides* f. *circinelloides*.
Penicillium roquefortii also known as *Penicillium roqueforti*.
Hansenula polymorpha also known as *Pichia angusta*.
Geobacillus stearothermophilus - former name *Bacillus stearothermophilus*.
4-Phytase also known as 6-phytase.

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[5] 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.

[5] 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.

3. REFERENCES

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Attachment 3

Review of the nomenclature of currently permitted enzymes and their approved sources

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 International Union of Biochemistry and Molecular Biology (IUBMB) allocates a four-digit EC number, the first three digits define the reaction catalysed and the fourth is a unique identifier (serial number). Each enzyme is also assigned a systematic name that uniquely defines the reaction catalysed (IUBMB 1992).

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.

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 a 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.

It is noted that the assessment and approval of enzymes are applied to the commercial enzyme preparation. For practical purposes the enzyme preparation is usually standardised to one main enzyme activity, (and it is usually related to that specific IUBMB number) but other activities are present. It is the commercial enzyme preparation, which includes all its enzymatic activities as well as the source organism, which is taken into consideration when assessing the safety of this preparation.

Detailed consideration of enzymes listed as permitted processing aids

Table to clause 15: 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 above listed enzymes, derived from animals, were first formerly included in a single standard when Standard A16 of the Australian *Food Standards Code* was gazetted 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.

Table to clause 16 - 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
Retain the entry for Bromelain.

FSANZ had originally suggested amending the entry for bromelain to 'stem bromelain' since the original approval of the enzyme was sourced from pineapple stem (*Ananas comosus*).

Further consideration

Following the final round of public consultation, several industry submissions and submissions from international-based industry associations recommended retaining the original entry. Respondents were concerned that changing the current permission to specify stem bromelain did restrict the present permissions and may unintentionally remove permission for bromelain currently being used.

Retaining this entry provides industry with continued flexibility associated with this entry. There is no change in the associated permitted source organisms and those approved are considered safe for this purpose. This revised recommendation also takes into account jurisdictional reluctance to amend entries where consequential trade disruptions and associated resource implications may occur.

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.

Industry argued 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. This suggestion was supported.

Further consideration
Following circulation to the EAG, industry suggested the permission of source for this enzyme is extended from malted cereals to ungerminated barley. This suggestion was not supported 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.

Table to clause 17 - 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

It is a widely used term that is consistent with the current bounds of IUBMB Enzyme Nomenclature.

Aminopeptidase EC [3.4.11.1]	*Lactococcoccus 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
Retain the entry for 'aminopeptidase' as a result of advice sought from industry indicating that a name change to 'leucyl aminopeptidase' may unintentionally remove permission for aminopeptidases currently being used.

It was initially proposed that the name be amended to a more specific name but advice from the EAG was sought on the implication to industry of this suggested name change. At the time no comment was provided.

FSANZ consideration

Since the release of the Draft Assessment Report (DAR), an industry association and manufacturer recommended to retain the current entry for aminopeptidase. Both respondents were concerned that changing the current permission to specify leucyl aminopeptidase did restrict the present permissions and may unintentionally remove permission for aminopeptidases currently being used.

Retaining this entry provides industry with continued flexibility and potential product innovation associated with this entry. There is no change in the associated permitted source organisms and those approved are considered safe for this purpose. This revised recommendation also takes into account jurisdictional reluctance to amend entries where consequential trade disruptions and associated resource implications may occur.

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'.

The term 'endo-arabanase', 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'.

The minor change to the entry is made 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 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
Retain the entry for carboxyl proteinase and the associated approved source of micro-organisms.

FSANZ originally suggested removing this entry and replacing it with those enzymes from the above list that industry nominate as being used in Australia and New Zealand. FSANZ was also seeking official technical documentation to substantiate this advice.

Following the final round of public consultation, several industry submissions and submissions from international-based industry associations recommended retaining this entry. Industry submitters did not offer any suggested replacement enzyme entries.

It was noted that the IUBMB list of enzymes is continually expanding as new enzymes are being discovered and identified.

Post DAR consideration

Submissions from industry and several international-based industry associations recommended retaining this entry. They do not see benefit of updating the names to align with revised IUBMB nomenclature as it complicates the positive enzyme permissions, not simplifying it.

FSANZ has reconsidered its recommendation to delete the above entries as it was dependant on industry providing appropriate alternatives. FSANZ accepts the industry recommendation to retain the above entries, noting that this will maintain a more simplified positive list of permitted enzymes for food use. It is also recognised that this recommendation, to retain the listing for this entry (as well as the terms bromelain, metalloproteinase and serine proteinase, see their discussions as well), will provide industry with flexibility for product innovation associated with these entries. FSANZ also recognises that there is no change in the associated permitted source organisms and those approved are considered safe for this purpose

Finally, jurisdictional comment included reluctance to delete entries where approval status of a product may have consequential trade disruptions and associated resource implications

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.

The simpler entry is retained 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.

The current entry is retained as it combines specificity with 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'.

The simpler entry is made 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'.

The IUBMB listed 'common name' which is still relatively simple, but is a more accurate description of the function of the enzyme and is the preferred name.

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
Retain the word 'Hemicellulase' and therefore no change.

Initially FSANZ suggested removing the general term 'hemicellulase' while remaining consistent with IUBMB Enzyme nomenclature.

Further consideration

Following release of the DAR submissions from industry and two prominent industry associations recommended the retention of the term 'hemicellulase' as it can still be used as a primary descriptor of this type of enzyme preparation that tends to have multicomponent properties. Suggestions were also made to include in the Editorial note an entry describing the multicomponent nature of hemicellulases.

FSANZ notes that the assessment and approval of enzymes are applied to the commercial enzyme preparation. For practical purposes the enzyme preparation is usually standardised to one main enzyme activity, (and it is usually related to that specific IUBMB number) but other activities are present. FSANZ agrees that it is the commercial enzyme preparation, which includes all its enzymatic activities as well as the source organism, which is taken into consideration when assessing the safety of this preparation.

FSANZ accepts the industry comments and has reconsidered its position so that it now recommends retaining the term hemicellulase. However it does not consider it necessary to include an editorial note describing the multicomponent nature of hemicellulase.

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 the two different genetically modified *Aspergillus oryzae* were gazetted in December 1997 and May 1997 respectively.

Recommendation - Table to clause 17
(a) Delete the words 'or xylanase' to leave the entry 'Hemicellulase endo-1,4-β-xylanase'.
(b) Amend the entry for permitted sources of micro-organism by replacing the Greek letter 'α' with the Greek letter 'β'. The latter corrects an editorial error.

As for the previous entry, FSANZ suggested removing the general term 'hemicellulase' while remaining consistent with IUBMB Enzyme nomenclature.

Further consideration

The term 'hemicellulase' has been kept for the same reasons outlined in the previous entry: 'hemicellulase endo-1,3-β-xylanase'.

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
Retain the entry 'Hemicellulase multicomponent enzyme'

Initially FSANZ suggested to remove the term 'hemicellulase multicomponent enzyme' and replacing it with Endo-1,4-β-mannanase to align the EC number with the corresponding IUBMB Enzyme name.

Further consideration

The term 'hemicellulase' has been kept for the same reasons outlined in the previous entries for 'hemicellulase endo-1,3-β-xylanase' and 'hemicellulase endo-1,4-β-xylanase'.

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.

Amend the name of the source micro-organism *Saccharomyces lactis* to *Kluyveromyces lactis*.

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. This approach to listing in the Code was considered 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*'

The simplest amended entry was supported taking into consideration the consistency with IUBMB enzyme nomenclature and accuracy of the name with respect to the function of the enzyme. Also amended 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
Retain the entry for metalloproteinase and the associated approved source of micro-organisms.

FSANZ consideration

Please refer to discussion presented under carboxyl proteinase for the justification on retaining the current entry for metalloproteinase.

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 industry about a typographic error, which has since been addressed. Reference was also made 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'.

Recommendation – Editorial note
Insert the sentence:
'4-phytase is also known as 6-phytase according to IUBMB nomenclature.'

FSANZ consideration
Following the DAR, 2 submissions from industry included specific advice supporting the change in nomenclature from 6-phytase to 4-phytase but also requested that a footnote be included to clarify any confusion the change of name may incur.

The advice was noted and agreed with and an entry in the Editorial note included to reflect that '4-phytase is also known as 6-phytase according to IUBMB nomenclature'.

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
Retain the entry for 'Polygalacturonase or Pectinase multicomponent enzyme'.

In response to the DAR, one of the industry-based technical associations have sought to retain the words 'or pectinase multicomponent enzyme' for the entry for polygalacturonase. They also suggest that an editorial note be included: 'pectinase products are multicomponent enzymes that contain polygalacturonase, pectin lyase and pectin esterase as main components.' to clarify the meaning of multicomponent enzyme

FSANZ consideration
FSANZ notes that the assessment and approval of enzymes are applied to the commercial enzyme preparation. For practical purposes the enzyme preparation is usually standardised to one main enzyme activity, (and it is usually related to that specific IUBMB number) but other activities are present. FSANZ agrees that it is the commercial enzyme preparation, which includes all its enzymatic activities as well as the source organism, which is taken into consideration when assessing the safety of this preparation.

FSANZ sees merit in retaining the words 'or pectinase multicomponent enzyme', as it was originally assessed and approved as a commercial enzyme preparation known as 'pectinase multicomponent enzyme'. However FSANZ does not agree to amending the editorial note to include a statement 'Pectinase products are multicomponent enzymes that contain polygalacturonase, pectin lyase and pectin esterase as main components', as it is unnecessary.

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
Retain the entry for serine proteinase and the associated approved sources of micro-organisms.
Replace the entry of *Bacillus lentus* with *Bacillus halodurans*.
Add an Editorial note saying '*Bacillus lentus* is the former name of *Bacillus halodurans'*.

FSANZ consideration

Please refer to discussion presented under carboxyl proteinase for the justification on retaining the current entry for serine proteinase.

As part of Novozymes submission to this issue they advised that the source organism *Bacillus lentus* should be replaced by *Bacillus halodurans.* Explanatory documentation provided by Novozymes sufficiently justifies changing the microbiological source to read *Bacillus halodurans* rather than *Bacillus lentus.* It is therefore agreed to amend the source organism but not to including an editorial note as this is correcting a technical error rather than updating a name.

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.

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

In response to the DAR, an enzyme association advised that this enzyme was typically called transglucosidase in commerce. They supported the use of the IUBMB common name but suggested this be coupled with a statement in the Editorial Note to this effect.

FSANZ recommends retaining the original name listed, and therefore negating the need to amend the Editorial note.

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 2004 (Amendment 70).

Recommendation
No change.

Updating the names of source organisms and other issues associated with the Editorial note

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*.

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

FSANZ confirmed industry advice that *B. amyloliquefaciens* is now a separate species to *B. subtilis* and suggested that the statement '*Bacillus subtilis* covers the strain known under the name *Bacillus amyloliquefaciens*' is no longer required in the editorial note. Additional information was sought from the enzyme industry to confirm which entries of *B. subtilis* should actually read *B. amyloliquefaciens*.

Advice from received from both the EAG and in submissions to the DAR which addressed the above issue. AMFEP provided the following detailed justification:

- Both species are able to produce the enzymes listed.
- Both species are safe and suitable hosts for the enzymes listed.

- Enzymes that were evaluated before 1987 were all classified as derived from *Bacillus subtilis.* It is not possible to determine in retrospect which of these would presently be classified as derived from *Bacillus amyloliquefaciens.*
- Present producers may not have in all cases re-classified the source organism.
- Most positive enzyme lists outside Australia/New Zealand, including JECFA, still list *Bacillus subtilis* based on the pre-1987 classification. Thus, in order to reflect current international standards, both organisms should be mentioned.

Further consideration

FSANZ notes that the current entry in the editorial note effectively recognises *B. amyloliquefaciens* as a permitted source organism wherever *B. subtilis* is listed. Taking into account the above justifications and the current Code FSANZ has reconsidered its previous conclusions and recommends: With the exception of genetically modified enzymes, all entries that are sourced from *B. subtilis* should also include a listing for *B. amyloliquefaciens* as a permitted source organism.

FSANZ further agrees to include an appropriate editorial note to help explain the change in relationship of *Bacillus amyloliquefaciens* to *Bacillus subtilis*.

Recommendation – Table to clause 17
With the exception of the genetically modified enzymes, all entries that are sourced from *B. subtilis* should also include a listing for *B. amyloliquefaciens* as a permitted source organism.

Insert *Bacillus amyloliquefaciens* for the following listed enzymes:
α–Acetolactate decarboxylase
α-Amylase
β-Amylase
β-Glucanase
Hemicellulase endo-1,4-β-xylanase
Hemicellulase multicomponent enzyme
Metalloproteinase
Pullulanase
Serine proteinase

Recommendation – Editorial note

Replace the sentence:
'*Bacillus subtilis* covers the strain known under the name *Bacillus amyloliquefaciens*.' with:
'*Bacillus amyloliquefaciens* is a separate species to *Bacillus subtilis.*

<u>*Bacillus stearothermophilus*</u>

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.

Saccharomyces fragilis as the former name of *Kluyveromyces fragilis*

An issue arising from late comment from an industry association advised that the source organism, *Saccharomyces fragilis,* is replaced by *Kluyveromyces fragilis*.

FSANZ consideration

Following further investigation FSANZ has ascertained that the names *Saccharomyces fragilis* and *Kluyveromyces fragilis* are both superseded by *Kluyveromyces marxianus.* FSANZ therefore recommends that the entries for *Saccharomyces fragilis* be amended to reflect this update and that the editorial note is suitably amended.

Recommendation – editorial note
Amend the sentence: '*Saccharomyces fragilis* is also known as *Kluyveromyces fragilis* and *Kluyveromyces marxianus var. marxianus.*'
Insert the sentence: '*Saccharomyces fragilis* and *Kluyveromyces fragilis* are the former names for *Kluyveromyces marxianus.*'

Streptomyces mobaraense

An issue arising from late comment from an industry association advised that we amend the entry of the source organism, *Streptomyces mobaraense,* to reflect its correct name *Streptomyces mobaraens*is.

FSANZ consideration

This suggestion is supported and it is recommended that the entry for *Streptomyces mobaraense* is amended to reflect its correct name, *Streptomyces mobaraens*is.

Recommendation – Table to clause 17
For the enzyme transglutaminase amend the name of the permitted source organism from *'Streptomyces mobaraense',* to its correct name '*Streptomyces mobaraens*is'.

Recommendation – Editorial note
Amend the last word in the sentence: '*Streptoverticillium mobaraense* is the former name for *Streptomyces mobaraense*' to read '*mobaraens*is'

Kluyveromyces lactis

An industry issue received in a submission was that the entry for *Saccharomyces lactis* should be updated to *Kluyveromyces lactis* to reflect current nomenclature.

FSANZ consideration

This suggestion is supported and it is recommended that the entry for *Saccharomyces lactis* (as a source for the enzyme β –galactosidase is amended to reflect its correct name, *Kluyveromyces lactis.* The editorial note also needs to be amended to reflect this as well.

Recommendation – Table to clause 17

Recommendation – Editorial note
Amend the sentence: *'Saccharomyces lactis* is also known as *Kluyveromyces lactis' to 'Kluyveromyces lactis* – former name *Saccharomyces lactis'*.

Reference

NC-IUBMB (2006) *Enzyme Nomenclature – recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology on the nomenclature and classification of enzymes by the reactions they catalyse.* Nomenclature Committee - International Union of Biochemistry and Molecular Biology http://www.chem.qmul.ac.uk/iubmb/enzyme/. Accessed 2006-2007.

Attachment 4

Business Cost Calculator Report

Proposal P276 - Review of Processing Aids (Enzymes)

Problem:	Standard 1.3.3 regulates the use of processing aids in food manufacture, prohibiting their use in food unless there is a specific permission within the standard. Matters being considered part of the review include: (1) Safety of currently approved enzymes, and by-products of enzymatic reactions and the guidelines for the safety assessment of enzymes. (2)Nomenclature used for enzymes and source organisms. (3) Enzymes not currently used in Australia and New Zealand. (4) Other issues raised by submitters after first round of public consultation
Objective:	Provide appropriate permissions for enzymes used in Australia and New Zealand.

Policy Options

Option Name	Quickscan Result
Status Quo	FALSE
Review clauses regulating processing aids and make amendments to the code as required	FALSE

Compliance Cost Summary

Option Name: Status Quo
Businesses Affected: N/A

Type	Cost per Business	Total Cost of Regulation
N/A	N/A	N/A

Option Name: Review clauses regulating processing aids and make amendments to the code as required
Businesses Affected: N/A

Type	Cost per Business	Total Cost of Regulation
N/A	N/A	N/A

Caution should be used comparing options and interpreting results over time. The Business Cost Calculator does not estimate the future values of ongoing costs. Refer to the User Guidelines for further information.

This report contains summaries of compliance costs only. An assessment on the compliance cost in itself does not provide an answer to which policy option is the most effective and efficient one. Rather, it provides information which needs to be considered alongside other relevant factors and issues when deciding between alternative policy options.

Attachment 5

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.

Round Two

Submitter organisation	Name
Food Technology Association of Victoria Inc.	David Gill
The New Zealand Food Safety Authority	Carole Inkster
Queensland Department of Health	Gary Bielby
Department of Health, SA	Joanne Cammans
NSW Food Authority	Jenine Ryle
DSM Food Specialties	Dick van Schouwen
Association of Manufacturers & Formulators of Enzyme Products (AMFEP)	Youri Skaskevitch
Danisco	Huub Scheres
Novozymes Australia Pty Ltd	Tony Bryan
Private	Ivan Jeray
Enzyme Technical Association	Anthony Pavel, Jr.

Submitter	Position	Comments
Food Technology Association of Victoria Inc.	Supports	Supports Option 2, to review clauses 15, 16 & 17 of Std 1.3.3.
The New Zealand Food Safety Authority	Supports	Supports Option 2, to review clauses 15, 16 & 17 of Std 1.3.3. Suggests retaining existing enzymes where there is some question about whether or not they are obsolete.
Environmental Health Unit of Queensland Health	Supports	Supports Option 2, to review clauses 15, 16 & 17 of Std 1.3.3.
Department of Health, SA	Supports	Supports Option 2, to review clauses 15, 16 & 17 of Std 1.3.3.
NSW Food Authority	Support with specific comments.	Supports Option 2, to review clauses 15, 16 & 17 of Std 1.3.3. But has also made specific comments: Concern that removal/alteration of current permissions do not unintentionally create trade disruptions with resource implications to both jurisdictions and industry. Commenting on the need for any editorial changes to the Code to be communicated to industry, especially small business to facilitate a smooth transition.
DSM Food Specialties	Support structure of standard & provide specific comment.	Provided very specific & technical comment: Support retaining the general entries for: Carboxyl proteinase; Metalloproteinase; Serine proteinase; and Hemicellulase.

Submitter	Position	Comments
		Not all existing enzyme proteins on the market are notified to IUBMB & therefore lack a specific dedicated EC number, but fall within the existing general entries like proteinase or hemicellulase or cellulase. That EC numbers be used as reference numbers but not limited to these EC numbers. Do not support the deletion of general entries such as amino peptidase, carboxyproteinase and serine proteinase and replacing them with more specific entries because this will cause some proteolytic enzymes to no longer be permitted. Do not support the deletion of hemicellulase pointing out that the same is not be applied to another multifunctional enzyme – cellulase. Name changes on enzymes activities have commercial drawbacks and that additional education/information is then required to educate Australian/New Zealand customers. Other issue Update the name for the micro-organism *Saccharomyces lactis* to *Kluyveromyces lacti* .for the entry for lactase.
Association of Manufacturers and Formulators of Enzyme Products (AMFEP)	No position stated.	Provided specific & technical comment which is summarised below: • It is difficult if not impossible to determine without error, which if any of the enzymes presently listed are no longer being used. Support leaving the list as is to allow for the potential marketing of enzymes from a variety of safe sources for the Australian & New Zealand food manufacturers to choose from. • Supports the listing of both species i.e. Bacillus subtilis and Bacillus amyloliquefaciens, since B. amyloliquefaciens is a separate species and not a strain of B. subtilis. • Keep footnotes for previous names of enzymes producing organisms. • Does not support changing our list to delete entries where IUBMB has reclassified an entry and provided a number of more specific entries and provides specific information to this effect. This complicates entries unnecessarily and does not offer any simplification of the positive list. Therefore Amfep proposes that the following entries be retained: carboxyl proteinase; Metalloproteinase; and serine proteinase, hemicellulase; bromelain and aminopeptidase. • Has no objection to renaming of 6-phytase to read 4-phytase but suggests for clarity that a footnote be included explaining that 'according to the IUBMB nomenclature, 4-phytase is also called 6-phytase. *Other issues:* • Provided technical clarification that enzymes act on specific substrates and therefore there is no need to question unintended by-products. • Provided detailed update of EU regulatory situation.

Submitter	Position	Comments
Danisco	No position stated.	Provided very specific & technical comment. In general support the opinions of ETA & AMFEP: • Impossible to identify obsolete enzymes from the positive list. • Both *Bacillus subtilis* and *B. amyloliquefaciens* should be listed as micro-organisms from which enzymes can be derived. • Current entries for proteases should be maintained. • Protein engineered enzymes should be exempt from GM labelling as the difference to their counterpart native enzyme is much smaller than differences between enzymes with the same IUBMB number. Specific comments: • Clarifying previous comments [p64 of DAR] attributed to Danisco and that they produce subtilisin from B. licheniformis. • That bromelain be replaced with both fruit and stem bromelain and that neither poses a safety issue. • Do not support name change from aminopeptidase to leucyl aminopeptidase as it is more restrictive and may change the regulatory status of currently approved products.
Novozymes Australia Pty Ltd		In general Novozymes supports comments and proposals made by AMFEP, ETA and JAFA. Provided additional specific & technical comments which are summarised below: **6-Phytase to 4-Phytase** Novozymes does not object to amending the current entry in the Code from 6-Phytase to 4-Phytase, on the condition that a footnote is added clarifying that 4-Phytase is also called 6-Phytase in other numbering systems and that they are equivalent. This request is based on the fact that 6-Phytase is mentioned in the IUB as an equivalent name for 4-Phytase and corresponds to numbering based on the reaction mode of the enzyme. The footnote in the Code would avert confusion. **Hemicellulase** Since hemicellulases are composed of xylanases, mannanases and arabinases (endo and exo) and their corresponding glycosidases, polygalacturonate hydrolase should be removed as it is a pectinase.
Ivan Jeray	Against enzymes from genetically modified sources.	Does not support the inclusion of GM substances as processing aids (enzymes) in Standard 1.3.3. States that GM substances have not been proved to be safe and that their consumption is not supported by Australian consumers. If enzymes produced from GMOs were approved for use in food, affected foods should be labelled accordingly, as consumers have a right to know that they are consuming genetically modified food.

Submitter	Position	Comments
Enzyme Technical Association (a trade association of companies that represents manufacturers and distributors of enzyme preparation in the US, Canada and Mexico)	No position stated.	Provided very specific & technical comment which are summarised below: ETA could not identify any obsolete processing aid enzymes and therefore recommended not to remove any enzymes from the list of processing aids. *Section 5.1* Supports the idea of FSANZ exempting enzymes from the planned safety review where they have been evaluated & approved by other international agencies. Supports the view of history of safe use and notes that 'Extensive review by recognised authorities have concluded that enzyme preparations from nontoxigenic, non-pathogenic organisms are safe to consume and that the primary safety concern is the safety of the production organism.' ETA is not aware of any case in a commercial food application where an enzyme has produced chemical products that ere a safety concern. *Section 5.2* No enzymes are identified as 'obsolete' and therefore no enzymes should be removed from the list. *Section 5.3.1* Does not support using the IUBMB as the primary nomenclature system and we should also consider nomenclature used by JECFA, EU, US and Japan. Suggest changes also retain reference of previous names or source organisms. *Section 5.5.1* Supports the conclusion that enzymes from genetically modified source organisms do not require labelling. Also that this exclusion also apply when the enzyme has been protein engineered. Other issues: Provided a more accurate description of the US regulation of processing aids/Enzymes. Both Bacillus subtilis and Bacillus amyloliquefaciens should be listed as micro-organisms from which enzymes can be derived. Entries for Carboxyl proteinase; Metalloproteinase; and Serine proteinase should be retained. Entry for hemicellulase should be retained with additional editorial note explaining what type of multicomponent enzyme hemicellulase is. Transglucosidase – for consistency change the name to 1.4-α-glucan 6-α-glucosyltransferase with a footnote explaining the change. For the entry for polygalacturonase retain the words pectinase multicomponent enzyme, and add editorial note 'pectinase products are multicomponent enzymes that contain polygalacturonase, pectin lyas and pectin esterase as main components.'