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

13-03
19 November 2003

INITIAL / DRAFT ASSESSMENT REPORT

PROPOSAL P283

WINEMAKING

DEADLINE FOR PUBLIC SUBMISSIONS to FSANZ in relation to this matter:
31 December 2003
(See 'Invitation for Public Submissions' for details)

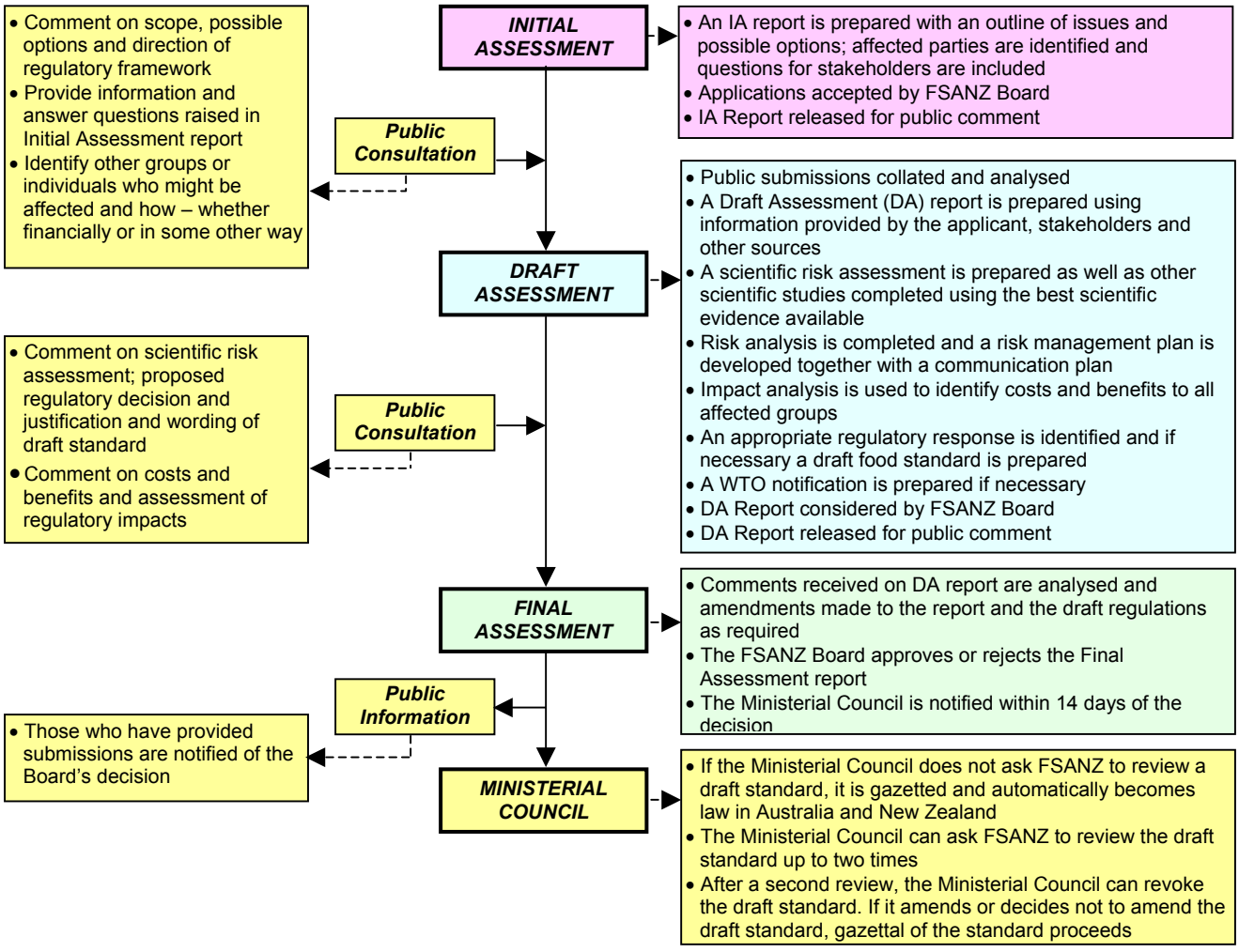
FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

FSANZ's role is to protect the health and safety of people in Australia and New Zealand through the maintenance of a safe food supply. FSANZ is a partnership between ten Governments: the Commonwealth; Australian States and Territories; and New Zealand. It is a statutory authority under Commonwealth law and is an independent, expert body.

FSANZ is responsible for developing, varying and reviewing standards and for developing codes of conduct with industry for food available in Australia and New Zealand covering labelling, composition and contaminants. In Australia, FSANZ also develops food standards for food safety, maximum residue limits, primary production and processing and a range of other functions including the coordination of national food surveillance and recall systems, conducting research and assessing policies about imported food.

The FSANZ Board approves new standards or variations to food standards in accordance with policy guidelines set by the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council) made up of Commonwealth, State and Territory and New Zealand Health Ministers as lead Ministers, with representation from other portfolios. Approved standards are then notified to the Ministerial Council. The Ministerial Council may then request that FSANZ review a proposed or existing standard. If the Ministerial Council does not request that FSANZ review the draft standard, or amends a draft standard, the standard is adopted by reference under the food laws of the Commonwealth, States, Territories and New Zealand. The Ministerial Council can, independently of a notification from FSANZ, request that FSANZ review a standard.

The process for amending the *Australia New Zealand Food Standards Code* is prescribed in the *Food Standards Australia New Zealand Act 1991* (FSANZ Act). The diagram below represents the different stages in the process including when periods of public consultation occur. This process varies for matters that are urgent or minor in significance or complexity.



INVITATION FOR PUBLIC SUBMISSIONS

FSANZ has prepared an Initial/Draft Assessment Report of Proposal P283; and prepared a draft variation to the *Australia New Zealand Food Standards Code* (the Code).

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

Written submissions are invited from interested individuals and organisations to assist FSANZ in preparing the Final Assessment for this Proposal. Submissions should, where possible, address the objectives of FSANZ as set out in section 10 of the FSANZ Act. Information providing details of potential costs and benefits of the proposed change to the Code from stakeholders is highly desirable. Claims made in submissions should be supported wherever possible by referencing or including relevant studies, research findings, trials, surveys etc. Technical information should be in sufficient detail to allow independent scientific assessment.

The processes of FSANZ are open to public scrutiny, and any submissions received will ordinarily be placed on the public register of FSANZ and made available for inspection. If you wish any information contained in a submission to remain confidential to FSANZ, you should clearly identify the sensitive information and provide justification for treating it as commercial-in-confidence. Section 39 of the FSANZ Act requires FSANZ to treat in-confidence, trade secrets relating to food and any other information relating to food, the commercial value of which would be, or could reasonably be expected to be, destroyed or diminished by disclosure.

Submissions must be made in writing and should clearly be marked with the word 'Submission' and quote the correct project number and name. Submissions may be sent to one of the following addresses:

Food Standards Australia New Zealand
PO Box 7186
Canberra BC ACT 2610
AUSTRALIA
Tel (02) 6271 2222
WWW.FOODSTANDARDS.GOV.AU

Food Standards Australia New Zealand
PO Box 10559
The Terrace WELLINGTON 6036
NEW ZEALAND
Tel (04) 473 9942
www.foodstandards.govt.nz

Submissions should be received by FSANZ **by 31 December 2003**.

Submissions received after this date may not be considered, unless the Project Manager has given prior agreement for an extension.

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

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

Further Information

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

Food Standards Australia New Zealand
PO Box 7186
Canberra BC ACT 2610
AUSTRALIA
Tel (02) 6271 2222
www.foodstandards.gov.au

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Executive Summary and Statement of Reasons

This Proposal seeks amendments to update and align the provisions regulating wine in the Code with those of other wine producing countries with which Australia and New Zealand trade in wine. These amendments are relevant to the ratification of the multi-lateral wine agreement on trade in wine, the World Wine Trading Group Agreement on Mutual Acceptance of Oenological Practices (MAA), by Australia and New Zealand, who signed the MAA in December 2001.

A requirement for the ratification of the MAA is confirmation, following a comparison of regulations from signatory countries, that there are no regulatory impediments to the free flow of product across borders. FSANZ has recently identified a number of amendments needed to the Code to ensure, as far as is possible, that wine from all signatory countries complies with the Code. The proposed amendments cover winemaking practices that are permitted in one or more of Australia's and New Zealand's co-signatories to the MAA: Argentina, Canada, Chile, and the USA.

Delay in ratification of the MAA may adversely affect Australia's trade in wine to the USA. The USA Government have made it clear that all wine imported into the USA from countries that have not signed and ratified the MAA will be subject to a stringent certification system from early in 2004.

Department of Foreign Affairs and Trade (DFAT) has recently advised the Department of Agriculture, Fisheries and Forestry (DAFF) that all legislative changes, including Code amendments, must be complete before Australia can ratify the MAA. New Zealand is in a similar situation. Discussions with representatives from the New Zealand High Commission, Attorney-General's Department, Office of International Law, DAFF and FSANZ have confirmed that the quickest route to ratification of the MAA is through the Code amendments process, rather than through other legislative changes.

It is not possible under FSANZ's regulatory requirements to process the required Code amendments by the time the USA certification requirements are likely to come into force. However with Board agreement to consider the Assessment Reports out-of session, amendments may be gazetted two to three months earlier than standard timeframes would allow. It is also proposed to use section 36 of the FSANZ Act, omitting one round of public comment, on the basis that to do so will not significantly adversely affect the interests of any person or body. Ministers also will be asked to consider these amendments as a priority so that gazettal of any Board approved Code changes may proceed as quickly as possible.

This Initial/Draft Assessment Report evaluates the issues associated with these permissions in the Code and recommends draft variations to the Code for further consideration in view of a round of public comment before the Final Assessment Report is prepared.

The proposed amendments to the Code are:

- inclusion for use in wine of gum arabic, calcium ascorbate, sodium ascorbate and sodium erythorbate, which are food additives already approved for use at GMP levels in most processed foods;

- inclusion for restricted use in wine of the food additives ethyl maltol and maltol, (flavourings and flavour enhancers), with proposed use limited to wine made with non-*Vitis vinifera* grapes[1]; and
- inclusion of argon, ammonium sulphite and the enzyme urease, as new processing aids in the Code.

The main objective of this assessment is to ensure that the proposed amendments to the standards in the Code that regulate the manufacture of wine do not adversely affect public health and safety.

Recommendations and Statement of Reasons

In making its recommendations on these matters FSANZ has considered:

- public health and safety issues associated with the proposed amendments; and
- issues associated with the technological justification for the proposed amendments.

At Initial/Draft Assessment FSANZ recommends that item 14.2.2, of Schedule 1, Standard 1.3.1 – Food Additives, be amended to include permission for:

- gum arabic (INS 414), with maximum level of use to be limited by good manufacturing practice (GMP);
- calcium ascorbate (INS 302), with maximum level of use to be limited by GMP;
- sodium ascorbate (INS 301) with maximum level of use to be limited by GMP;
- sodium erythorbate (INS 316) with maximum level of use to be limited by GMP;
- ethyl maltol (INS 637), with maximum level of use to be limited to 100 mg/kg and not permitted for use in wine made with *Vitis vinifera* grapes; and
- maltol (INS 636), with maximum level of use to be limited to 250 mg/kg and not permitted for wine made with *Vitis vinifera* grapes.

At Initial/Draft Assessment FSANZ recommends that Standard 1.3.3 – Processing Aids be amended as follows:

- include argon in the table to clause 3 as a generally permitted processing aid for use in all foods, with maximum level of use to be limited by GMP;
- include urease in the table to clause 17 as a permitted enzyme of microbial origin, with maximum level of use to be limited by GMP; and
- include ammonium sulphite in the table to clause 18 as a permitted microbial nutrients and microbial nutrient adjuncts, with maximum level of use to be limited by GMP.

[1] *Vitis vinifera* is the vine species that produces over 99 percent of the world's wines today. It is native to Europe as well as East and Central Asia, but it has been planted all over the world. There are estimated to be thousands of varieties of this species, some of the best known being cabernet sauvignon, chardonnay, chenin blanc, merlot, pinot noir, riesling, sauvignon blanc, syrah, and zinfandel.

Reasons for these recommendations are that the proposed amendments:

- would raise no public health and safety concerns because FSANZ's safety assessments conclude that the food additives and processing aids are considered to be safe at the levels of use proposed;

- are technologically justified because FSANZ's food technology reports conclude that the food additives and processing aids perform a technological purpose at the levels of use proposed, and, in the case of the processing aids, do not perform a technological function in the final food;

- will promote consistency between domestic and international food standards by improving consistency of the Code with the winemaking provisions of other countries with which Australia and New Zealand trade in wine; and

- will promote an efficient and internationally competitive food industry by removing regulatory obstacles to the ratification of the World Wine Trade Group Mutual Acceptance Agreement (MAA), to which Australia and New Zealand are signatories.

1. Introduction

Australia and New Zealand signed the World Wine Trade Group Mutual Acceptance Agreement (MAA) in December 2001. A requirement for the ratification of the MAA was confirmation, following a comparison of regulations from signatory countries, that there were no regulatory impediments to the free flow of product across borders. The detailed documentation with full regulatory requirements, including additive and processing aid permissions, has only recently become available, and FSANZ has identified a number of amendments needed to the Code to ensure, as far as is possible, that wine from all signatory countries is Code compliant.

2. Regulatory problem

As part of the ratification process for the MAA, FSANZ has recently been provided with information about the regulatory requirements of the signatory countries. While this has allowed FSANZ to identify a number of Code amendments that are needed to help ensure that wine from signatory countries is compliant, the late arrival of this information has meant that it will not be possible for FSANZ to make these amendments before the date for ratification given the current schedule of Board meetings.

The Department of Foreign Affairs and Trade (DFAT) has recently advised the Department of Agriculture, Fisheries and Forestry (DAFF) that all legislative changes, including Code amendments, must be complete before Australia can ratify the MAA. Up until now, DAFF and the Winemakers' Federation of Australia (WFA) believed that ratification was possible whilst Code amendments were in progress.

2.1 Consequences of late ratification of MAA

Delay in ratification of the MAA may adversely affect Australia's trade in wine to the USA. The USA Government has made it clear that all wine imported into the USA from countries that have not signed and ratified the MAA will be subject to a stringent certification system from early in 2004. New Zealand is in a similar situation.

2.2 Quickest route to ratification of the MAA

Discussions with representatives from the New Zealand High Commission, Attorney-General's Department, Office of International Law, DAFF and FSANZ have confirmed that the quickest route to ratification of the MAA for both countries is through the Code amendments process, rather than through other legislative change.

It is not possible under FSANZ's regulatory framework to process the required Code amendments by the time the USA certification requirements are likely to come into force. However with Board agreement to consider the Assessment Reports out-of session, amendments may be gazetted two to three months earlier than standard timeframes would allow. The Board agreed to use section 36 of the FSANZ Act, omitting one round of public comment, on the basis that to do so does not significantly adversely affect the interests of any person or body. Ministers also will be asked to consider these amendments as a priority so that gazettal of any Board approved Code changes may proceed as quickly as possible.

3. Objective

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

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

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

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

One of the objectives of this Proposal is to ensure that the necessary Code amendments are put in place in order to remove the regulatory obstacles to the ratification of the MAA. However the main objective of this assessment is to ensure that the proposed amendments to the standards in the Code that regulate the manufacture of wine (see Section 5 for details) do not adversely affect public health and safety. The assessment will also need to be consistent with the other section 10 objectives of the FSANZ Act.

4. Background

4.1 Work Plan Classification

This Proposal has been rated as Category of Assessment 2 (level of complexity) and placed in Group 1 on the FSANZ standards development Work Plan.

Ratification of the MAA is dependent on wine regulations in the Code aligning with the wine regulations of our co-signatories in the MAA. This Proposal seeks to assess the suitability of amendments to the Code that would align its winemaking regulations with those of Australia and New Zealand's co-signatories of the MAA: Argentina, Canada, Chile and the USA.

Because of the importance of ratifying the MAA in the shortest possible time, there is a strong public interest relating to the effective administration in the food regulatory system that justifies this proposal being placed in Work Plan Group 1. It is also proposed to implement section 36 of the FSANZ Act, omitting one round of public comment, on the basis that to do so does not significantly adversely affect the interests of any person or body.

Further details about the Work Plan and its classification system are given in *Information for Applicants* at www.foodstandards.gov.au.

5. Relevant Issues

5.1 Permission to use gum arabic (acacia) as a food additive

Gum arabic or acacia gum or arabic gum, is the dried gummy exudate from tropical and sub-tropical *Acacia senegal* trees and related Acacia species. It is used in winemaking in many countries for stabilisation as it prevents the formation of cloudiness and deposits by stopping unstable colloid particles from aggregating in clarified wine.

Gum arabic (CAS No: 9000-01-5) consists mainly of high-molecular weight polysaccharides and their calcium, magnesium and potassium salts, which on hydrolysis yield arabinose, galactose, rhamnose and glucuronic acid. Items of commerce may contain extraneous materials such as sand and pieces of bark, which must be removed before use in food.

Gum arabic is currently listed as a generally permitted food additive in Schedule 2 of Standard 1.3.1 – Food Additives. Schedule 2 Food Additives are also generally permitted processing aids. The New Zealand *Food Regulations 1984*, (revoked in December 2002) permitted the use of gum arabic in wine. However the Code does not permit Schedule 2 food additives to be used for wine made in accordance with Standard 2.7.4 unless they are specifically listed in Schedule 1.

Gum arabic is approved by the Office International de la Vigne et du Vin (OIV) for use in wine. It is permitted for use in wine made in the European Union (EU), in South Africa and in the USA. Australia's Agreement with the EU on trade in wine also permits the use of gum arabic in wines made in the EU for sale in Australia.

Gum arabic is classified by the Joint Expert Committee of Food Additives (JECFA) as an emulsifier, thickening agent and stabiliser. According to the latest evaluation of gum arabic by JECFA in 1989, its ADI is 'not specified'[2], if used according to, and limited by, good manufacturing practice (GMP).

5.1.1 Evaluation and impact analysis

Gum arabic is already a generally permitted food additive (Standard 1.3.1, Schedule 2). However, these generally permitted Schedule 2 food additives are not permitted for use in wine made in accordance with Standard 2.7.4 unless specifically listed in Standard 1.3.1, Schedule 1. Listing in Schedule 2 means gum arabic has been assessed and is considered to be safe for use in food at GMP levels and therefore that there are no public health or safety issues associated with extending its use wherever there is a need to use it in processed foods.

The use of gum arabic as a food additive in wine is a widely accepted winemaking practice and amending the Code to permit its use for wines made in accordance with Standard 2.7.4 will benefit all affected parties and align the joint wine standard more closely with those of other wine producing countries with which Australia and New Zealand trade in wine.

[2] 'ADI not specified' is used where a food substance has been evaluated by JECFA as having a very low toxicity on the basis of the available data and total dietary intake of the substance.

5.1.2 Preferred approach

At Draft Assessment FSANZ proposes to amend Standard 1.3.1, schedule 1, item 14.2.2 to include permission for gum arabic (INS 414) with maximum level of use limited by GMP.

5.2 Permission to use calcium ascorbate, sodium ascorbate and sodium erythorbate as food additives

Ascorbic acid and erythorbic acid are currently included in Standard 1.3.1, schedule 1, item 14.2.2 for use in wine made in accordance with Standard 2.7.4. Ascorbic acid is commonly known as Vitamin C. Erythorbic acid, also known as isoascorbic acid, is an isomer of ascorbic acid that has similar chemical properties but with less of the vitamin activity. The function of ascorbic acid and erythorbic acid function in wine is as an antioxidant or preservative. This use is common if not universal in the domestic winemaking regulations of countries throughout the world.

The calcium and sodium salts of ascorbic acid are permitted for use in Canada and the sodium salt of erythorbic acid is included in the winemaking regulations of Canada and Argentina. The salts have the same function in wine as the parent food acids but have slightly different solubility and acidity. Once dissolved, the salts dissociate into the ionised form of the food acid and calcium or sodium ions. It would be difficult if not impossible to determine whether the acid or the salt were used because calcium and sodium ions are present from other constituents in wine. Permission to use the salts as well as the food acids would provide winemakers with convenient alternative chemical forms of the food acids.

5.2.1 Evaluation and impact analysis

Calcium ascorbate, sodium ascorbate and sodium erythorbate are already generally permitted food additives (Standard 1.3.1, schedule 2). However these generally permitted Schedule 2 food additives are not permitted for use in wine made in accordance with Standard 2.7.4 unless specifically listed in Standard 1.3.1, Schedule 1. Listing in Schedule 2 means that these food additives have been assessed and considered to be safe for use in food at GMP levels and therefore that there are no public health or safety issues associated with extending their use wherever there is a need to use them in processed foods.

The use of these salts of ascorbic acid and erythorbic acid as food additives in wine is a widely accepted winemaking practice and amending the Code to permit their use for wines made in accordance with Standard 2.7.4 will benefit all affected parties and align the joint wine standard more closely with those of other wine producing countries with which Australia and New Zealand trade in wine.

There are no health or safety reasons for not approving inclusion of these food acid salts in the Code. Without permission in the Code, imported wines containing them could not legally be sold in Australia or New Zealand. Providing permission for their use not only would align the Code with the domestic winemaking provisions of our trading partners but would also provide winemakers with the convenience of being able to use alternative chemical forms of the already permitted food acids.

5.2.2 Preferred approach

At Draft Assessment FSANZ proposes to amend Standard 1.3.1, schedule 1, item 14.2.2 to include permission for calcium ascorbate (INS 302), sodium ascorbate (INS 301) and sodium erythorbate (INS 306), with their maximum levels of use limited by GMP.

5.3 Permission to use ethyl maltol and maltol as food additives

Ethyl maltol and maltol are used as flavourings or flavour enhancers. Ethyl maltol, 2-Ethyl-3-hydroxy-4-pyrone (CAS no: 4940-11-8) has a molecular weight of 140.14. Maltol, 3-hydroxy-2-methyl-4-pyrone, (CAS No: 118-71-8) has a molecular weight of 126.11.

The USA winemaking regulations permit the use of these flavour enhancers, but not in wine made from *Vitis vinifera* grapes[3]. Since, ninety-nine per cent of winemaking grapes are of the *V. vinifera* variety, this means in practice that most US-made wine is not permitted to contain ethyl maltol or maltol. Neither of these substances is included in the winemaking regulations of the major wine producing countries with which Australia and New Zealand trade in wine, i.e., the EU, Argentina, Chile or Canada.

Standard 1.3.1, Schedule 1, item 11.4 – Tabletop Sweeteners includes permission for maltol and ethyl maltol with maximum level of use limited by GMP. Both substances can also be used as flavourings or as ingredients of flavourings and are thus permitted in many processed foods.

The joint WHO/FAO expert Committee of Food Additives (JECFA) has determined Acceptable Daily Intakes (ADIs) of 0-2 mg/kg bw for ethyl maltol and 0-1 mg/kg bw for maltol. For those wines that are permitted to contain these substances, the limit of use in the USA regulations is 100 mg/kg in the case of ethyl maltol and 250 mg/kg in the case of maltol.

5.3.1 Evaluation and impact analysis

The use of flavourings and flavour enhancers is not widely recognised or practised in winemaking countries round the world. The flavour enhancers, ethyl maltol and maltol, are permitted for use in wine in the USA but not for wine made from *Vitis vinifera* grapes. Noting that wine is made almost exclusively from *V. vinifera* grapes, the Winemakers' Federation of Australia (WFA) advises that most wine imported from the USA is likely to be made from *V. vinifera* grapes, in which these flavour enhancers are not permitted. WFA further advises that according to their USA colleagues, wines made from non-*V. vinifera* grapes are just not a commercial proposition and they do not know of anyone still using these substances.

Given the rarity of wines made from non-*V. vinifera* grapes, it is unlikely that wine containing ethyl maltol or maltol will be imported into Australia and New Zealand. However, because it is possible in theory that such wines will come in, permission for these flavour enhancers should be included in the Code (provided that such permission raises no public health or safety concerns) in order that these wines can be legally sold in New Zealand and Australia so that the ratification of the MAA can be completed.

[3] The vine species that produces over 99 percent of the world's wines today. It is native to Europe as well as East and Central Asia, but it has been planted all over the world. There are estimated to be thousands of varieties of this species, some of the best known being cabernet sauvignon, chardonnay, chenin blanc, merlot, pinot noir, riesling, sauvignon blanc, syrah, and zinfandel.

WFA also advises that all grapes used for winemaking in Australia and New Zealand are of the *V. vinifera* variety. This means that including permission in the Code for these substances, but only for wines made with non-*V. vinifera* grapes, would mean that no Australian or New Zealand made wine would be permitted to contain these substances.

5.3.2 Estimated dietary intake of ethyl maltol and maltol from wine

Dietary surveys show wine intake in Australia and New Zealand to be almost identical. For people over 15 years of age, the mean intakes of wine per day (including all red, white, fortified and cider wines) are 338 millilitres in Australia (mL) and 287 mL in New Zealand. The 95th percentile intakes for wine are 795 mL in Australia and 747 mL in New Zealand.

The assumption that all wine consumed in Australia and New Zealand will contain ethyl maltol and maltol, and at the maximum permitted levels, leads to a vast overestimate of the likely dietary intake of these substances from wine. Only a small number of USA wines are made from non-*V. vinifera* grapes and of these, very few, if any, would contain ethyl maltol or maltol, let alone at the maximum permitted levels. It is also highly unlikely that any of these wines will be imported into Australia and New Zealand.

On enquiry from WFA, USA winemakers were unable to identify any winemakers who use ethyl maltol or maltol and so levels of usage and the percentage of wines in which they are used cannot be determined accurately. However, using recent Australian imports of USA wine as a guide for estimating the amount of wine that may contain ethyl maltol and maltol we have:

- USA wine imports into Australia were 338,000 litres in 2001-2002; and
- Australian total wine consumption was 420 million litres in 2001-2002.

That is, 0.08 per cent of Australian wine consumption was USA-produced.

If all wine imported from the USA contained ethyl maltol and maltol at the maximum limits permitted by the USA regulations then the intake at the 95th percentile of wine consumption in Australia and New Zealand of ethyl maltol would be approximately 0.04 to 0.05 per cent of the JECFA determined ADI and the intake for maltol would be approximately 0.22 per cent of the JECFA determined ADI.

If we assume that 1 per cent of all USA wine imports were to contain ethyl maltol and maltol at the maximum permitted limits, then 0.0008 per cent of all wine consumed in Australia would contain ethyl maltol and maltol and would contribute 0.0004 per cent to 0.0005 per cent of the ADI for ethyl maltol and 0.002 per cent to 0.003 per cent of the ADI for maltol in high consumers of wine (95th percentile consumers).

Clearly the likely contribution of wine to the overall intake of ethyl maltol and maltol would be negligible given current consumption and import patterns and would remain insignificant even if the proportion of USA wine consumed were to increase by several orders of magnitude.

In summary, the only source of ethyl maltol and maltol in wine will be from USA imports and the volume of wine containing these substances is likely to be negligible. The estimated dietary intake from wine for ethyl maltol and maltol is also negligible.

FSANZ therefore proposes to permit the use of these flavour enhancers in wine at the same levels as are permitted in the USA regulations but only in those wines made from non-*Vitis vinifera* grapes.

5.3.3 Preferred approach

At Draft Assessment FSANZ proposes to amend Standard 1.3.1, schedule 1, item 14.2.2 to include permission for ethyl maltol (INS 637), with a maximum permitted level in the final food of 100 mg/kg, and maltol (INS 636), with a maximum permitted level in the final food of 250 mg/kg.

FSANZ also proposes that maltol and ethyl maltol not be permitted for use in wine made with *Vitis vinifera* grapes.

5.4 Permission to use argon as a processing aid

Argon is a colourless, odourless, inert gas. It is heavier than carbon dioxide or nitrogen, more readily displacing oxygen than these other gases. Therefore it provides a better protective gas cover over wine during production, thus better preventing oxidation of wine and the growth of unwanted bacteria and yeast.

Argon is not currently included in the Code for use during the manufacture of any food, including wine.

Argon is approved for use in wine as a processing aid by the Office International de la Vigne et du Vin (OIV). It is permitted for use in wine made in the EU. Australia's Agreement with the EU on trade in wine also permits the use of argon for wines made in the EU for sale in Australia and also for wines made in Australia for sale in the EU.

In addition, argon is listed in the *Codex inventory of all compounds used as processing aids* (Appendix A), as a propellant and packaging gas, as are carbon dioxide and nitrogen (Codex Committee on Food Additives and Contaminants 1999); the initial Inventory of Processing Aids was adopted by the Codex Alimentarius Commission at its 18th Session in 1989, from whence it had been sent to all Member Nations and Associate Members of FAO and WHO as an advisory text.

5.4.1 Safety assessment of argon

Argon is an inert noble gas, which is a normal component of atmospheric air, and is colourless, odourless and tasteless, non-corrosive, non-flammable and non-toxic. It is stable as a gas. Since argon is a gas (boiling point: -185.9 ˚C), exposure through ingestion is unlikely. Argon can be absorbed into the body by inhalation. On loss of containment this gas can cause suffocation by lowering the oxygen content of the air in confined areas.

From the available information, it is concluded that the use of argon as a processing aid in food would pose no public health and safety risk.

5.4.2 Technological justification for use of argon

The food technology report on the use of argon as a processing aid in wine (at Attachment 2) recommends that argon should be approved for use in winemaking as a processing aid since it has a technological purpose during wine production or processing, including bottling, as a covering gas that displaces air and oxygen.

5.4.3 Evaluation and impact analysis

There are no public health or safety issues associated with the use of argon as a processing aid during winemaking and packaging. The use of argon as a processing aid during wine production is a widely accepted practice in other wine producing countries.

Amending the Code to permit the use of argon for wines made in accordance with Standard 2.7.4 will benefit all affected parties and align the Code's provisions regulating wine more closely with those of the wine producing countries with which Australia and New Zealand trade in wine.

Due to its complete chemical inertness, there are no public health and safety issues associated with the use of argon as a processing aid for any food. Therefore, provided there is technological justification for its use, argon would be a suitable processing aid for use during the manufacture of any food. Providing a general permission for the use of argon, rather than just for wine, will prevent the need for future Applications to amend the Code to permit the use of argon during manufacture of various individual foods.

5.4.4 Preferred approach

At Draft Assessment, FSANZ proposes to amend Standard 1.3.3, table to clause 3 to include argon as a generally permitted processing aid for use in all foods, which includes wine made in accordance with Standard 2.7.4.

5.5 Permission to use urease as a processing aid

The Code does not currently permit the use of urease for wine made in accordance with Standard 2.7.4.

The European Commission has requested that the enzyme urease be added to Annex 1 (2) of the *Agreement between Australia and the European Community on Trade in Wine, and Protocol* (EC 94/184), which specifies permitted winemaking practices for wines originating in the EU for sale in Australia.

Urease was accepted by the OIV for use in winemaking in 1995. It is permitted for use during winemaking in the USA and in the EU.

Urease is an enzyme that reduces the levels of naturally occurring urea in wine by facilitating the hydrolysis of urea to ammonia and carbon dioxide. Lowering the urea level reduces the formation of ethyl carbamate.

Foods and beverages such as bread, cheese, milk, olives, soy sauce and yoghurt can contain a measurable concentration of ethyl carbamate following fermentation. When administered at high doses in animal studies, ethyl carbamate has the potential to be carcinogenic. The ethyl carbamate dose levels used in animal studies were much higher than the levels expected in fermented foods. Alcoholic beverages including wine can also contain a measurable concentration of ethyl carbamate, which may be significantly higher than that in other foods because urea, in combination with ethanol, may directly form ethyl carbamate.

Canada has placed maximum limits on the concentration of ethyl carbamate that a wine/wine product can contain. Furthermore the UK MAFF and USA FDA/BATF have also undertaken to reduce significantly the concentration of ethyl carbamate in wine, and have recommended that wine importers initiate routine analyses for ethyl carbamate in wine.

5.5.1 Safety assessment of urease

The safety assessment of urease from *Lactobacillus fermentum* (at Attachment 3) concluded that:

- the source organism is a common constituent of many foods and a commensal of the human gut flora;
- the enzyme preparation complies with international specifications;
- no antimicrobial activity was demonstrated in culture medium in which *Lactobacillus fermentum* was tested against six known common pathogenic organisms;
- in a sub-acute study in rats, no adverse effects were observed at the highest dose;
- the NOEL (no observable effect level) from the sub-acute gavage study is 2000 mg per kg bw per day; and
- the enzyme preparation produced no evidence of genotoxic potential in *in vitro* assays.

From the available information, it is concluded that the use of urease as a processing aid in food would pose no public health and safety risk.

5.5.2 Technological justification for use of urease

The food technology report (at Attachment 4) concludes that the use of urease sourced from *Lactobacillus fermentum* is technologically justified as a wine processing aid since it has a technological purpose to reduce the concentration of urea in wine so limiting the formation of ethyl carbamate as a processing aid in wine.

5.5.3 Evaluation and impact analysis

The source organism, *Lactobacillus fermentum*, is a non-toxic, non-pathogenic organism that is part of the normal gut flora in humans, rats, cows and other animals. It is also a common constituent of the bacteria found in soil. Its common presence in the normal gut flora of humans and animals has led to its widespread use as a probiotic for treatment and prevention of a variety of ailments such as diarrhoea, and urogenital tract infections where pure cultures of *L. fermentum* are used to re-establish normal bacterial flora and prevent the regrowth of pathogenic organisms.

L. fermentum is present commonly in many kinds of fermented European foods and in the Indian fermented food 'dosa'. The organism has been consumed by man traditionally as part of his daily food. Bacterial cultures of the organism are already permitted in the Code for use in fermented milk products. Since the organism itself is already approved for food use, there are no additional public health and safety issues associated with its use to produce an enzyme.

The safety assessment and food technology reports (at Attachments 3 and 4 respectively) for urease concluded that the use of urease is technologically justified and would raise no public health and safety concerns

Urease is permitted for use during winemaking in the USA, the EU, Argentina and Chile. Inclusion of urease in the Code would align Australia's and New Zealand's winemaking regulations with those of the countries with which we trade in wine. The use of urease, as outlined above has the potential to reduce significantly the levels of urea in wine, leading to reduced production of ethyl carbamate.

5.5.4 Preferred approach

At Draft Assessment FSANZ proposes to amend Standard 1.3.3, table to clause 17 to include permission for urease.

5.6 Permission to use ammonium sulphite as a processing aid

Ammonium sulphite is permitted as a yeast nutrient in the EU and in Chile. It is also listed in Annex 1 of Australia's Agreement with the EU on trade in wine.

Standard 1.3.1, Schedule 1, item 14.2.2 includes permission for the use of sulphur dioxide and six of its related salts as preservatives in wine. For public health and safety reasons there is a maximum permitted level for the combined total of these sulphites in the final wine of 250 mg/kg (for wines with less than 35 g/L of residual sugars) and of 400 mg/kg (for wines with more than 35 g/L of residual sugars).

The use of ammonium sulphite as a yeast nutrient is not currently permitted in the Code. Its proposed use as a yeast nutrient would also mean that ammonium sulphite is utilised by the yeast as fermentation proceeds. GMP limits would require its use to be limited to a quantity sufficient for yeast nutrient and no more. The quantity required for yeast nutrition would be far less than that required for a preservative function. Any sulphite left in the final food from use as a yeast nutrient would in any case be included in any measurement of sulphites in the final food and the Code's requirement for the mandatory declaration of sulphites would inform consumers with a sensitivity to sulphites of its presence in the wine.

5.6.1 Evaluation and impact analysis

A significant portion of the population is sensitive to sulphites in foods. This common adverse effect is reflected in the Code's requirement for the mandatory declaration on the label of any food that has 10 mg/kg or more of added sulphites. Also for health and safety reasons the Code sets a maximum limit for total sulphites in the final wine.

The proposed use of ammonium sulphite as a processing aid would not result in an increase in the Code's maximum permitted level for total sulphites. Any remaining sulphite from its use as a processing aid would be included in any measurement of total sulphites. Its use as a yeast nutrient would account for only a small fraction of the total sulphites used as a preservative.

Without permission in the Code, imported foods made with ammonium sulphite as a processing aid could not legally be sold in Australia or New Zealand. Providing permission for its use not only would align the Code with the winemaking provisions of our trading partners but would also provide winemakers with the convenience of being able to use an additional yeast nutrient.

The Code's mandatory labelling requirements would inform consumers of the presence of sulphites and the maximum limit set in the Code for total sulphites in wine would not be changed.

5.6.2 Preferred approach

At Draft Assessment FSANZ proposes to amend Standard 1.3.3, Table to clause 18 to include ammonium sulphite.

6. Regulatory Options

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

The following regulatory options are available for this Application:

Option 1 Approve all the proposed changes to the wine regulations in the Code.

Option 2 Not approve any of the proposed changes to the wine regulations in the Code.

Option 3 Approve some but not all of the proposed changes to the wine regulations in the Code.

7. Impact Analysis

7.1 Affected Parties

The affected parties to this Application include those listed below:

1. wine producers, importers and exporters in Australia, New Zealand and worldwide;

2. wine consumers in Australia and in New Zealand;

3. Australian State and Territory and New Zealand Government enforcement agencies that enforce food regulations; and

4. enforcement agencies in countries importing wine made in Australia or New Zealand.

7.2 Impact Analysis

The costs and benefits relating to the proposed amendments and issues raised in submissions that are associated with these costs and benefits are analysed under the relevant issue-specific headings in Section 5 above.

8. Consultation

FSANZ decided, pursuant to section 36 of the FSANZ Act to omit to invite public submissions in relation to the Proposal prior to making a Draft Assessment. FSANZ now invites written submissions for the purpose of the Final Assessment under s.17(3)(c) of the FSANZ Act and will have regard to any submissions received.

FSANZ made its decision under section 36 because it was satisfied that omitting to invite public submissions prior to making a Draft Assessment would not have an adverse effect on anyone's interests.

Section 63 of the FSANZ Act provides that, subject to the *Administrative Appeals Tribunal Act 1975*, an application for review of FSANZ's decision to omit to invite public submissions prior to making a Draft Assessment, may be made to the Administrative Appeals Tribunal.

8.1 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 widely accepted international standards for winemaking. Amending the Code to allow the proposed changes to wine regulation is likely to assist trade in wine, especially in countries with which Australia has existing agreements on trade in wine, because the proposed changes are consistent with those countries' domestic wine regulations. There does not appear therefore to be a need to notify the WTO.

9. Conclusion and Recommendation

In making its recommendations on these matters FSANZ has considered:

- public health and safety issues associated with the proposed amendments; and
- issues associated with the technological justification for the proposed amendments.

At Initial/Draft Assessment, FSANZ recommends that item 14.2.2, of Schedule 1, Standard 1.3.1 – Food Additives, be amended to include permission for:

- gum arabic (INS 414), with maximum level of use to be limited by good manufacturing practice (GMP);

- calcium ascorbate (INS 302), with maximum level of use to be limited by GMP;
- sodium ascorbate (INS 301) with maximum level of use to be limited by GMP;
- sodium erythorbate (INS 316) with maximum level of use to be limited by GMP;
- ethyl maltol (INS 637), with maximum level of use to be limited to 100 mg/kg and not permitted for use in wine made with *Vitis vinifera* grapes; and
- maltol (INS 636), with maximum level of use to be limited to 250 mg/kg and not permitted for wine made with *Vitis vinifera* grapes.

At Initial/Draft Assessment, FSANZ recommends that Standard 1.3.3 – Processing Aids be amended as follows:

- include argon in the table to clause 3 as a generally permitted processing aid for use in all foods, with maximum level of use to be limited by GMP;
- include urease in the table to clause 17 as a permitted enzyme of microbial origin, with maximum level of use to be limited by GMP; and
- include ammonium sulphite in the table to clause 18 as a permitted microbial nutrients and microbial nutrient adjuncts, with maximum level of use to be limited by GMP.

Reasons for these recommendations are that the proposed amendments:

- would raise no public health and safety concerns because FSANZ's safety assessments conclude that the food additives and processing aids are considered to be safe at the levels of use proposed;
- are technologically justified because FSANZ's food technology reports conclude that the food additives and processing aids perform a technological purpose at the levels of use proposed, and, in the case of the processing aids, do not perform a technological function in the final food;
- will promote consistency between domestic and international food standards by improving consistency of the Code with the winemaking provisions of other countries with which Australia and New Zealand trade in wine; and
- will promote an efficient and internationally competitive food industry by removing regulatory obstacles to the ratification of the World Wine Trade Group Mutual Acceptance Agreement (MAA), to which Australia and New Zealand are signatories.

10. Implementation and review

FSANZ recommends that the effective date for the proposed amendments be from the date of gazettal.

ATTACHMENTS

1. Draft variations to the *Australia New Zealand Food Standards Code.*
2. Food technology report for argon.
3. Safety Assessment report for urease.
4. Food technology report for urease.

Attachment 1

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

To commence: on gazettal

[1] ***Standard 1.3.1*** *of the Australia New Zealand Food Standards Code is varied by inserting in* Schedule 1, *under item* 14.2.2 Wine, sparkling wine and fortified wine, *the following entries –*

302	Calcium ascorbate	GMP		
637	Ethyl maltol	100	mg/kg	Wine made with other than *Vitis vinifera* grapes only
414	Gum arabic	GMP		
636	Maltol	250	mg/kg	Wine made with other than *Vitis vinifera* grapes only
301	Sodium ascorbate	GMP		
316	Sodium erythorbate	GMP		

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

[2.1] *inserting in the* Table to clause 3 –

Argon	

[2.2] *inserting in the* Table to clause 17 –

Urease EC [3.5.1.5]	*Lactobacillus fermentum*

[2.3] *inserting in the* Table to clause 18 –

Ammonium sulphite

Attachment 2

Food technology report for argon

Argon (Ar) is colourless, odourless, inert, monoatomic gas (being one of the noble elements, group O or VIIIA of the Periodic Table). Other inert noble gases in this group are helium and neon. Noble gases are characterised by having an entirely filled electronic outer p subshell, which is the reason they are inert. Argon's atomic number is 18 and it has an atomic weight of 39.948. It is found at low levels in air. It is normally obtained from the liquefaction and separation of air. Its abundance is 93.4 µL/L in dry air. Argon's density at Standard Temperature and Pressure (STP: 0°C, 1 atmosphere pressure) is 1.78 mg cm^{-3} compared to 1.25 mg cm^{-3} for nitrogen. Its solubility in water at 20°C is 33.6 cm^{-3}/kg (mL/L), which is greater than nitrogen but a lot less than carbon dioxide[1, 2].

Argon is one of three gases (the others are carbon dioxide and nitrogen) that the wine industry wishes to use to displace air (oxygen) during wine production and bottling. The use of such gases is to displace oxygen, thereby limiting deleterious oxidation of wine and preventing the growth of unwanted bacteria and yeast during wine production. Argon is the heaviest of the three gases so is best able to displace oxygen.

Argon is more expensive and is more soluble in water (and wine) than nitrogen but it has the advantage of being heavier than nitrogen so can displace air (oxygen) and so acts as an inert blanket gas better than nitrogen. Which displacement gas wine producers use will depend on the job they wish it to do and the balance of advantages and disadvantages.

Argon is a permitted processing aid for winemaking in various international organisations, including Codex (*Codex inventory of all compounds used as processing aids*, 1989), the Office International de la Vigne et du Vin (OIV) and the European Community (contained in Annex 1 of the *Agreement between the European Community and Australia on trade in wine*).

Argon is an inert gas, which if used in winemaking would not be considered a food additive since it has no function in the final food and does not meet any of the technological functions listed in Schedule 5 of Standard 1.3.1 of the *Australia New Zealand Food Standards Code*. Argon is technologically justified for use in winemaking as a processing aid because since it has a technological purpose during wine production or processing including bottling, as a covering gas that displaces air (oxygen), and does not perform this function in the final food.

References:

1. Greenwood N N and Earnshaw A Chemistry of the Elements 1984 Pergamon Press New York pp 1042-1045.

2. The Merck Index (13th Ed) 2001 Merck & Co. Inc. Whitehouse Station NJ.

Attachment 3

Safety assessment report for urease

1. Introduction

The objective of adding urease at the end of the fermentation process for making wine is the reduction of the urea content by conversion via hydrolysis to CO2 and NH3. If excess urea is formed in wine it will combine with the ethanol in wine during the storage and ageing period to form ethyl carbamate, which has the potential for carcinogenity when administered in high doses in animal tests, the presence of which in wine is undesirable. Urea can react with ethanol to form ethyl carbamate under certain circumstances. An important feature is that this reaction tends to need heat in order to form considerable amounts of ethyl carbamate. Groups of foods were this reaction might occur comprises wine, sake and probably bread. Ethyl carbamate can also be formed through reactions where urea is not required.

2. The source (production) organism – *Lactobacillus fermentum*

The safety of the production organism is an important consideration in the safety assessment for enzymes used as a processing aid.
In application A474 the approval is sought for the use of urease from a non-genetically modified *Lactobacillus fermentum* as a processing aid.

Lactobacillus fermentum has been described as a 'common intestinal indigenous bacteria'[4] that is 'commonly found in the digestive tracts of pigs and rodents and also present in man'[5]. It is also described as a "harmless bacteria" in a study using *L. fermentum* to 'treat a chronic infectious condition by the oral administration of a certain strain of lactobacillus'[6].

L. fermentum has been used as a starter culture in cheese preparation [7,8], an Ethiopian fermented food called Tef [9], Nigerian fermented foods Fufu and Ogi [10,11], African maize product Mawe [12] and has been shown to play an important role in the fermentation of soy sauce [13]. *L. fermentum* has also been described as part of the 'bacterial flora of samples from the process at malt whiskey distillery'[14].

In summary, the source organism is used in a range of foods and from the available data there are no public health safety concerns.

[4] Clinical Experimental Immunology (1999) – 118(2) : 261-267
[5] Plasmid (1997) – 37(3) : 199-203
[6] Scandinavian Journal of Infectious Disease (1996) – 28(6) : 615-619
[7] International Industrial Biotechnology (1988) – 8(4) : 36-37
[8] International Journal of Food Science and Technology (2000) – 35(6) : 577-581
[9] Journal of Food Science (1985) – 50(3) : 800-801
[10] International Journal of Food Microbiology (2002) – 72(1/2) : 53-62
[11] Journal of Applied Bacteriology (1988) - 65(6) : 449-453
[12] Tropical Science (1999) – 39(4) : 220-226
[13] Korean Journal of Applied Microbiology and Biotechnology (1999) – 27(2) : 113-117
[14] Journal of the American Society of Brewing Chemists (2003) – 61(1) : 10-14

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

2.1 Antibiotic activity

Antibiotic activity of the cultured broth of *Lactobacillus fermentum* IFO 14511. Study Director: Y Sumino, Central Research Division, Takeda Chemical Industries Ltd., Japan, 30 October, 1987.

Study design
Lactobacillus fermentum IFO 14511 was cultivated statically in a test tube containing 10 ml of the production medium for acid urease. After 2 days, samples were taken and placed on agar plates containing *Micrococcus luteus IFO 12708, Bacillus subtilis NIHJ PCI 219, Staphylococcus aureus FDA 209P, Escherichia coli NIHA JC-2, Pseudomonas aeruginosa IFO 3080, or Saccharomyces cerevisiae IFP 0209*. The plates were examined for growth inhibition of the test microorganism.

Result and conclusion
No inhibition of any of the six test microorganisms was observed (data not shown).

3. Purity of enzyme preparation and proposed specifications

Historically, enzymes used in food processing have been found to be non-toxic, and the main toxicological consideration is in relation to possible contaminants. The production organism in this case is non-toxic and non-pathogenic. The detailed specifications from the source to which the preparation was found to conform are shown in Table 1. This is consistent with the recommended purity specifications for food-grade enzymes[15,16].

Table 1. Complete specification of urease preparation

Criteria	Specification
Urease activity (U/mg)	> 5
Total viable count (cfu/g)	Negative by test
Aerobic count (cfu/g)	Not more than 5 X 10^4
Total coliforms (cfu/g)	Negative by test
Salmonella	Negative by test
Production strain	Negative by test
Antibacterial activity	Negative by test
Heavy Metals as Pb	Not more than 30 ppm
Arsenic	Not more than 2 ppm
Lead	Not more than 10 ppm
Loss on drying	Not more than 10%

[15] Joint FAO/WHO Expert Committee on Food Additives (JECFA), 2001. General specifications and considerations for enzyme preparations used in food processing. FAO Food and Nutrition Paper 52, Add. 9, pp. 37-39.
[16] National Academy of Sciences, Food and Nutrition Board, Committee on Food Chemical Codex. 1996. *Food Chemical Codex*, 4th edition, National Academy Press, Washington DC.

4. Evaluation of the submitted studies

Four toxicological studies were submitted in support of this application. These were:

- an acute toxicity study in mice and rats;
- a 28-day sub-acute oral toxicity study in rats;
- a modified mutagenic potential assay and modified bacterial mutagenicity assay; and
- a sex-linked recessive lethal test in *Drosophila melanogaster.*

4.1 Acute study

Acute toxicity of acid urease producing bacteria (viable cells of *Lactobacillus Fermentum*) in male mice and rats. Study Director: S Chiba, Central Research Division, Takeda Chemical Industries, Japan. Report no. X-74-8. 25 January 1988.

Test material	Acid urease producing bacteria (viable cells of Lactobacillus fermentum; lot no AU-62; $5x10^9$ cells/g; freeze-drying)
Vehicle material	Distilled water for oral routes and saline for the other routes.
Test Species	Jcl:ICR male mice and Jcl:Wistar male rats (2 animals/dose)
Dose	31.3-250 x 10^8 cells/kg for oral administration, 3.9-250 x 10^8 cells/kg for subcutaneous and intraperitoneal administration.
GLP/guidelines	not reported.

Groups of 2 male mice and rats received single doses of acid urease producing bacteria administered orally by gavage, intraperitoneally, or subcutaneously. The animals were observed for 14 days post-dose.

After oral administration no clinical signs or mortality were observed following any of the tested doses. Subcutaneous and intraperitoneal administration resulted in decreased loco motor activity and respiratory depression at most dose levels and mortality was observed in both mice and rats at doses > 31.3 x 10^8 cells/kg after intraperitoneal administration and > 62.5 x 10^8 cells/kg after subcutaneous administration.

4.2 Sub-acute toxicity

Four-week subacute oral toxicity study of AU-62 in rats. Study Director: R. Nagata, Shin Nippon Biomedical Laboratories, Ltd, Kagoshima, Japan. Report no. SBL 00-62. 12 October 1987.

Test material	AU-62 (crude preparation of acid urease), 6.0 U/mg
Control and vehicle material	Sterile water
Test Species	SPF Crj : CD (SD) rats 10 males and females per test dose; administration by gavage
Dose	0, 200, 600, 2000 mg urease /kg bw per day
GLP/guidelines	signed GLP and quality assurance statement; Guideline not specified

Study conduct
Four groups of rats (10/sex/group) were treated with urease by gavage at 0, 200, 600 or 2000 mg/kg bw per day for 28 days.

Clinical observations were recorded daily. Bodyweight and food consumption were recorded weekly; urinalysis in 5 animals/sex/group in week three of treatment; ophthalmology performed on animals in the control and high dose group in week three, and haematology and blood biochemistry was performed at the end of treatment. At the end of the study, all animals were sacrificed and necroscopy performed (gross examination, organ weights). Histopathology on selected organs was performed in the control and high dose group.

Results
No mortality and clinical signs were observed during treatment of urease. Food consumption, body weight, ophthalmology, and urinalysis were not adversely affected by treatment. The red blood cell count and haemoglobin concentrations were higher in males at 2000 mg/kg bw/day (7 and 4.5% increase as compared to controls, respectively), however these changes were not associated with any other haematological or histopathological findings, and therefore not considered to be treatment related. No other treatment related effects were observed in haematology and biochemistry. Necropsy revealed no abnormal changes in all groups.

The NOEL was 2000 mg/kg bw per day, the highest dose tested.

4.3 Genotoxicity studies

Bacterial mutagenicity study on crude acid urease powder. Study Director: Y Sakamoto. Central Research Division, Takeda Chemical Industries Ltd., Japan, Report No. X-74-9. 28 January, 1988.

Test article
The test article, powder of crude acid urease (AU-62, Lot No 22T) was used. The activity was not specified.

Study design
Urease was examined for mutagenic activity in three different tests: 1) repair test (modified rec-assay), 2) reversion test (Ames test) and 3) Modified Ames test. The tests were not performed according to specified international guidelines.

For the repair test, 0.1 ml of each cell suspension of *Bacillus subtilis* H17 (rec^+) or M45 (rec^-) was mixed with top-agar and overlaid on a nutrient agar plate. These indicator plates were scored and when the radius of an inhibition zone on the M45 plate was 2 mm more than that on the H17 plate, the article was classified as positive in the rec-effect.

In the reversion test two strains of *Salmonella typhimurium* (TA98, TA100) and one strain of *Escherichia Coli* (WP2urvA) were used. Experiments were performed and without metabolic activation using liver S9 fraction from chemically pre-treated rats.
The study comprised of negative and positive controls. Experiments for estimation of mutant numbers were carried out in duplicates. Four doses of test substance were applied with 5 mg/plate as the highest dose level The sensitivity of the individual bacterial strains was confirmed by significant increases in the number of revertant colonies induced by diagnostic mutagens.

For the modified Ames test, two strains of *Salmonella typhimurium* (TA98, TA100) were used. Experiments were performed with or without metabolic activation using liver S9 fraction from chemically pre-treated rats. The study comprised of negative and positive controls with or without S9 metabolising system. Experiments for survival determination and estimation of mutant numbers were carried out in duplicates at each test point. Five doses of test substance were applied with 5 mg/plate as the highest dose level. The sensitivity of the individual bacterial strains was confirmed by significant increases in the number of revertant colonies induced by diagnostic mutagens (positive controls).

Results and conclusion

Test	*Test material*	*Concentration*	*Test object*	*Result*
repair test (*In vitro*)	crude acid urease	0, 125, 1250 µg/plate	*Bacillus subtilis* H17 (rec^+) and M45 (rec^-)	-ve
reversion test (Ames test)	crude acid urease	0, 625, 1250, 2500, 5000 µg/plate with and without S9 mix	*S. typhimurium* TA98, TA100,. *E. Coli* (WP2urvA)	-ve
modified Ames test	crude acid urease	0, 313, 625, 1250, 2500, 5000 µg/plate with and without S9 mix	*S. typhimurium* TA98, TA100	-ve

The mutagenicity assays produced negative responses under the conditions of the three tests performed.

Tests of mutagenicity *in vivo* of crude acid urease powder with Drosophila somatic systems. Study Director: Y Sakamoto. Central Research Division, Takeda Chemical Industries Ltd., Japan, Report No. X-74-10. 28 January, 1988.

Test article
The test article, powder of crude acid urease (AU-62, Lot No 22T) was used. The activity was not specified.

Study design
The first study was a Wing-hair spot test. For this test, three to four day old *Drosophila melanogaster* females with the genotype y; mwh jv and males with the genotype y; $Dp(1;3)sc^{J4}$, flr/TMi, Me ri sbd were paired (20 /group) and allowed to lay eggs. The parental flies were discarded 24 h later and the resulting eggs were allowed to develop to adulthood. The test article was orally administered at a dose of 25 or 50 mg/ml to larval flies during 96 h (larval stage). Adult flies were fixed and wings were sampled for spots with 3 or more mwh hairs and those with neighbouring mwh and flr clones.

The second test was a DNA-repair test. For this, *Drosophila melanogaster* consisting of sc z^1 $w^{+(TE)}$ $mei\text{-}9^a$ $mei\text{-}41^{os}$ males and C(1)DX, y f females were used. The genotypes represent X-chromosomes. Three to four day old females and males were paired (20/group) and allowed to lay eggs. The parental flies were discarded 24 h later and the resulting eggs were allowed to develop to adulthood. The test article was orally administered at a dose of 25 or 50 mg/ml to larval flies during 96 h (larval stage).

A decrease in the male to female ratio from the control ratio was considered to be a positive test. In both tests 2-(Furyl)-3-(5-nitro-2-fyryl)acrylamide (AF-2) was used as a positive control. The tests were not performed to specified international (OECD) guidelines.

Results and conclusion

The results of the Wing-hair spot test and DNA-repair test showed no abnormality. The positive control AF-2 gave the expected increase in frequency per wing of mutant clones and dose dependent reduction in male to female ratio from the control level. Therefore, under the conditions of the test, urease did not increase mutagenicity. However, since the test was not performed according specified OECD guidelines, the test has limited value for the safety assessment of urease.

5. Conclusion

The safety assessment of urease from *Lactobacillus fermentum* concluded that:

- the source organism is a common constituent of many foods and a commensal of the human gut flora;
- the enzyme preparation complies with international specifications;
- no antimicrobial activity was demonstrated in culture medium in which *Lactobacillus fermentum* was tested against six known common pathogenic organisms.
- in a sub-acute study in rats, no adverse effects were observed at the highest dose;
- the NOEL from the sub-acute gavage study is 2000 mg/kg bw per day; and
- the enzyme preparation produced no evidence of genotoxic potential in *in vitro* assays.

From the available information, it is concluded that the use of urease as a processing aid in food would pose no public health and safety risk.

Attachment 4

Food technology report for urease

Urease is an enzyme derived from *Lactobacillus fermentum,* which is a non-pathogenic, non-toxicogenic bacterium. The Enzyme Commission number for this enzyme is EC [3.5.1.5], while its CAS number is 9002-13-5.

One role of urease is to break down and reduce the concentration of urea by facilitating the hydrolysis of urea to ammonia and carbon dioxide.

It is an important enzyme for winemaking (and other alcoholic industries) since it can be used to reduce the concentration of urea, which in turn reduces the accumulation of a known carcinogen ethyl carbamate. Ethyl carbamate is commonly formed by the reaction of urea with carbon dioxide. Urea is a precursor of ethyl carbamate so any reduction in the concentration of urea helps reduce the formation of ethyl carbamate. It has been shown that wines that have had the enzyme urease added to them during winemaking produce a significantly lower concentration of ethyl carbamate[1].

The reaction urease catalyses is:

$$NH_2(CO)NH_2 + H_2O \xrightarrow{\text{urease}} CO_2 + 2\ NH_3$$

urea carbon dioxide and ammonia

Source Organism, *Lactobacillus fermentum*

Urease is produced by a culture fermentation process from *Lactobacillus fermentum*, which is a non-pathogenic, non-toxigenic organism. *Lactobacillus fermentum* is commonly present in many types of fermented European foods (including yoghurts) and in the Indian fermented food 'dosa'. The organism has been consumed by man traditionally as part of his daily food. It is also a normal inhabitant of the gastrointestinal tract of humans[2].

Lactobacillus fermentum is a lactic acid producing micro-organism and as such is approved in the *Australia New Zealand Food Standards Code* for the production of yoghurt, in Standard 2.5.3 – Fermented Milk Products.

Production of urease preparation

The production of the urease enzyme preparation is performed from a pure culture of *Lactobacillus fermentum* aseptically fermented in a medium containing only dextrose, casein digest, meat extract, yeast extract, sodium chloride, sodium acetate and manganese sulphate. The biomass is homogenised in 50% ethanol for several hours and the final suspension is dried to a powder. This procedure destroys all viable source organisms. The activity of the final enzyme preparation is adjusted by dilution with cellulose powder or dextrin[4].

International Regulations

Urease enzyme preparation sourced from *Lactobacillus fermentum* is considered generally recognised as safe (GRAS) by the USA FDA (21 CFR section 184.1924). It is produced from a pure culture fermentation process using materials that are considered GRAS and approved food additives. It is approved for use in wine to convert urea to ammonia and carbon dioxide. The FDA did not set a specific limit of urease treatment of wine but rather left it to 'good commercial practice' since treatment will be economically self-limiting due to the cost of the enzyme. The use of urease treatment of wine is approved to limit the levels of urea in wine so reducing the formation of ethyl carbamate in wine.

The EU also allows the use of urease to treat wine, under EEC Regulation No 3220/90 Annex V. This restricts the treatment to 75 mg of urease preparation per litre of wine, not exceeding 375 units urease per litre wine. After treatment, all residual enzyme activity must be eliminated by filtering the wine (pore size <1.0 μm).

Urease is permitted for use in winemaking in the European Union according to Annex IV 4(c) 'List of authorised oenological practices and processes' of *EC Council Regulation No 1493/1999 on the common organisation of the market in wine.* This regulation does not have a limit on use of the enzyme.

The EU has also petitioned Australia to permit the use of urease for wine making within *the Agreement between the European Community and Australia on Trade in Wine* (in 1999) which Australia agreed to according to the EU document (VI/7301/99-EU-EU).

The Office International de la Vigne et du Vin (OIV) has also accepted the use of urease sourced from *Lactobacillus fermentum* in winemaking under its Resolution Oeno 2/95, to reduce the level of urea in wine so reduce the formation of ethyl carbamate.

International Regulations Concerning the Concentration of Ethyl Carbamate in Wine

Canada introduced guideline that limited the concentration of ethyl carbamate in various alcoholic beverages in 1985. These include 30 μg/kg for table wines and higher levels for fortified wines, spirits and fruit brandies and liqueurs reflecting the smaller levels each of these are generally consumed.

In the USA in 1988 the FDA, the Wine Institute and the Association of American Vintners instituted a voluntary program of wine ethyl carbamate levels in post 1988 vintage. These levels for wines containing 14 % or less alcohol, not greater than 15 μg/L and for wines with greater than 14% alcohol, not greater than 60 μg/L.

Both the UK (Ministry of Agriculture, Fisheries and Food, MAFF) and the USA (FDA and Bureau of Alcohol Tobacco and Firearms) have also undertaken to significantly reduce the concentrations of ethyl carbamate in wine. They have recommended that wine importers initiate routine analyses for ethyl carbamate in wine.

There is only limited data available on ethyl carbamate levels of Australian wine. The Australian Wine Research Institute performed analyses on wine exported to Canada which found very low levels, ranging from 2.5-8.5 μg/L. These low levels were confirmed by survey results undertaken by Canadian authorities in 1994/5.

Conclusion

Use of the enzyme urease sourced from *Lactobacillus fermentum* is justified as a wine processing aid since it has a technological purpose to reduce the concentration of urea in wine so limiting the formation of ethyl carbamate.

References

1. Ough, CS Crowell, EA, Mooney LA 1988 Formation of ethyl carbamate precursors during grape juice fermentation. I. Additions of amino acids, urea and ammonia. *Am. J. Enol. Viti*. **39**: 243–249

2. European Commission, Scientific Committee on Food. Opinion on the use of urease prepared from Lactobacillus fermentum in wine production (10 December 1998).