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

6-07
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DRAFT ASSESSMENT REPORT

APPLICATION A605

YEAST MANNOPROTEINS AS A FOOD ADDITIVE FOR WINE

DEADLINE FOR PUBLIC SUBMISSIONS: 6pm (Canberra time) 14 November 2007
SUBMISSIONS RECEIVED AFTER THIS DEADLINE WILL NOT BE CONSIDERED
(See 'Invitation for Public Submissions' for details)

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

Executive Summary

Food Standards Australia New Zealand (FSANZ) received an Application from Laffort Services on 11 April 2007 seeking approval to use mannoproteins extracted from yeast cell walls as a food additive in wine to inhibit the crystallisation of potassium bitartrate.

A pre-market assessment is required before approval for use of food additives is granted in Australia and New Zealand. The Application seeks to vary Standards 1.3.1 – Food Additives and 4.5.1 – Wine Production Requirements (Australia only) of the *Australia New Zealand Food Standards Code* (the Code). If the Application is approved, a variation to Standard 1.3.4 – Identity and Purity will also be required to include an additional secondary source of specifications which contains a specification of the additive.

Yeast mannoproteins are added to wine as a food additive to inhibit the formation of potassium bitartrate crystals which are commonly formed in bottled wine. The presence of potassium bitartrate crystals in wine is not an issue related to safety or wine taste but rather one of aesthetics and consumer acceptability.

The yeast mannoproteins preparation would provide a more cost effective and efficient alternative to the two current stabilisation treatments used to prevent formation of these crystals in wine bottles. The currently available methods are cold stabilisation, which involves keeping wine at very low temperatures for a long period of time to promote early crystallisation of the tartrate (which is removed by filtration before bottling) and use of metatartaric acid which is approved in the Code as a food additive for this purpose.

Mannoproteins are yeast cell wall components that are proteins with large numbers of mannose groups (sugar units) attached. Mannoproteins are extracted from the cell walls of the common yeast *Saccharomyces cerevisiae* using an enzyme treatment. The enzyme used is permitted for use in food manufacture. The yeast mannoproteins are proposed to be added to wine in a concentration range of 100-300 mg/L, which is consistent with levels used internationally. Yeast mannoproteins also occur naturally in wine and many other foods.

Yeast mannoproteins are approved for use to stabilise wine in the European Union and Argentina. The Agreement between Australia and the European Community on Trade in Wine, and Protocol (1994) allows the use of preparations of yeast cell wall (up to a level of 400 mg/L) for Australian and European produced wine. The Organisation Internationale de la Vigne et du Vin (OIV) (International Organisation of Vine and Wine) has approved the use of the additive to stabilise wine (both tartrate and protein stabilisation). The OIV International Oenological Codex (OIV Codex) contains a specification for yeast mannoproteins in OIV Resolution Oeno 26/2004. The OIV Codex is an internationally accepted reference for wine related specifications. To address the specification of yeast mannoproteins it is recommended to add the OIV Codex as a secondary source in clause 3 of Standard 1.3.4.

A safety assessment was conducted to identify any potential public health and safety risks associated with the use of yeast mannoproteins as a food additive in winemaking. The assessment was based on data on the chemistry, production process, and intended use of the yeast mannoprotein preparation provided by the Applicant and obtained from the scientific literature. FSANZ concluded that there are no safety concerns based on the considerations listed below.

- There is a long history of human consumption of the yeast *S. cerevisiae*, primarily due to its use in baking and brewing.

- Yeast and yeast extracts are safely consumed as dietary and nutritional supplements by humans and animals.

- Mannoproteins released from wine yeast during fermentation are naturally present in wine.

- Yeast mannoproteins are digested as normal dietary proteins.

- The β-glucosidase exo-1,3 EC [3.2.1.58] enzyme preparation used to extract yeast mannoproteins is approved in the Code as a food processing aid.

- Product specifications indicate the yeast mannoprotein preparation does not contain chemical or microbiological contaminants above relevant limits.

Approving yeast mannoproteins to inhibit potassium bitartrate crystallisation in the Code would also provide permission for protein stabilisation (that is, limit the formation of protein derived haze). Yeast mannoproteins that stabilise proteins have a different molecular weight to those that are effective for tartrate stabilisation (~40 kDa for tartrate stabilisation and 32 kDa for protein stabilisation). However, the specification does not specify molecular weight so both fractions would be permitted if the OIV Codex is included in the Code as a source of specifications.

There are two regulatory options under consideration, to approve or not approve the use of yeast mannoproteins as a food additive to stabilise wine. Approval would benefit the wine industry, manufacturers and suppliers of alternative wine stabilisation technologies and consumers. No significant costs to government agencies or consumers have been identified.

The Draft Assessment concludes that approval of yeast mannoproteins as a food additive for wine stability treatment is appropriate as no public health and safety concerns have been identified and the use is technologically justified.

Purpose

The purpose of the Application is to vary the Code to permit the use of yeast mannoproteins as a food additive to stabilise wine. The additive achieves this by inhibiting the formation of potassium bitartrate crystals in bottled wine.

Preferred Approach

FSANZ recommends the proposed draft variations to Standards 1.3.1, 1.3.4 and 4.5.1 to approve the use of yeast mannoproteins as a food additive for wine stability treatment and to recognise the Organisation Internationale de la Vigne et du Vin (OIV) International Oenological Codex (Edition 2006) as a specification source.

Reasons for Preferred Approach

This Application has been assessed against the requirements for Draft Assessments in the *Food Standards Australia New Zealand Act 1991* (FSANZ Act). FSANZ recommends the proposed draft variations to Standards 1.3.1, 1.3.4 and 4.5.1 for the following reasons.

- A detailed safety assessment did not identify any public health and safety concerns.
- Use of the additive is technologically justified as an alternative treatment to the currently permitted and used additives and processes.
- No issues were raised in submissions to the Initial Assessment identifying any risks associated with the proposed approval of yeast mannoproteins.
- The impact analysis concluded that the benefits of permitting the use of yeast mannoproteins as a food additive outweigh any associated costs.
- The proposed variations are consistent with the section 18 objectives of the FSANZ Act.

Consultation

FSANZ invited public submissions on the Initial Assessment Report. Seven submissions were received; one opposed and six supported or tentatively supported the Application pending the outcome of the safety assessment. Issues raised in submissions are discussed in section 9 of this report.

FSANZ is seeking public comment on this Draft Assessment Report to assist in assessing the Application. Comments on, but not limited, to the following would be useful:

- any impacts (costs/benefits) of the proposed variations to Standards 1.3.1, 1.3.4 or 4.5.1;
- any public health and safety considerations associated with the proposed approval; and
- any other affected parties to this Application.

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INVITATION FOR PUBLIC SUBMISSIONS

FSANZ invites public comment on this Draft Assessment Report based on regulation impact principles and the draft variations 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 of this Application. Submissions should, where possible, address the objectives of FSANZ as set out in section 18 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 confidential commercial information. Section 114 of the FSANZ Act requires FSANZ to treat in-confidence, trade secrets relating to food and any other information relating to food, the commercial value of which would be, or could reasonably be expected to be, destroyed or diminished by disclosure.

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

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

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

Submissions need to be received by FSANZ by 6pm (Canberra time) 14 November 2007.

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

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

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

INTRODUCTION

Food Standards Australia New Zealand (FSANZ) received an Application from Laffort Services on 11 April 2007 seeking permission to use mannoproteins extracted from yeast cell walls as a food additive in wine to inhibit the crystallisation of potassium bitartrate. The Application seeks to vary Standards 1.3.1 – Food Additives and 4.5.1 – Wine Production Requirements (Australia only) of the *Australia New Zealand Food Standards Code* (the Code). If the Application is approved, a variation to Standard 1.3.4 – Identity and Purity will also be required.

Mannoproteins are yeast cell wall components that are proteins with large numbers of mannose groups (sugar units) attached. Mannoproteins are extracted from the cell walls of the common yeast *Saccharomyces cerevisiae* using an enzyme treatment.

Yeast mannoproteins are added to wine as a food additive to inhibit the formation of potassium bitartrate crystals which are commonly formed in bottled wine. The presence of potassium bitartrate crystals in wine is not an issue related to safety or wine taste but rather one of aesthetics and consumer acceptability. There are two current stabilisation treatments used to prevent formation of these crystals in wine bottles. The first is called cold stabilisation and it involves keeping wine at very low temperatures for a long period of time to promote early crystallisation of the tartrate (which is removed by filtration before bottling). The second method involves the use of metatartaric acid which is approved in the Code as a food additive for this purpose.

1. Background

1.1 Current Standard

Currently there is no approval for the use of mannoproteins extracted from yeast cell walls as a food additive for wine stabilisation in the Code. A pre-market assessment of the yeast mannoproteins as used in stabilising wine is required before the additive can be approved or used for this purpose in Australia and New Zealand. Standard 1.3.1 – Food Additives regulates the use of food additives in food manufacture, prohibiting their use in food unless there is a specific permission in the Standard. Food additive permissions for wine are listed in Standard 1.3.1 in Schedule 1 - Permitted uses of food additives by food type under category 14.2.2 Wine, sparkling wine and fortified wine. Standard 4.5.1 – Wine Production Requirements applies to the production of wine in Australia only. Additives permitted for use in wine production are listed in the Table to clause 3. Amendments to both standards will be required if this Application is successful. New Zealand wine is required to meet Standard 1.3.1 and Australian wine is required to meet Standards 1.3.1 and 4.5.1.

Metatartaric acid is currently approved in both standards; it can be used to provide short term inhibition of potassium bitartrate crystallisation in wine.

The yeast mannoproteins perform a stabilising function in wine. This is an approved function in Standard 1.3.1 listed in Schedule 5 Technological functions which may be performed by food additives.

In addition, Standard 1.3.4 ensures that substances added to food in accordance with the Code meet appropriate specifications for identity and purity of food additives, processing aids, vitamins and minerals and other added nutrients. If approved, a variation to Standard 1.3.4 – Identity and Purity will be required.

1.2 Background

Wine, in its normal state, is supersaturated with potassium bitartrate salts which can often crystallise out in wine bottles. Potassium bitartrate crystals that form in stored wine are not considered a taste quality issue but their presence may be aesthetically unpleasant to consumers. Some consumers may believe that their presence is an indicator of poor quality, so wine manufacturers try to limit the formation of potassium bitartrate crystals in bottled wine. Various wine making strategies are employed such as cold stabilisation (extended cold temperature storage for a period of time to force the crystallisation of the potassium bitartrate which is then removed before bottling) or the use of metatartaric acid which gives only relatively short term stability[1].

Historically, it was believed that wine, especially red wine, naturally contained macromolecules that act as protective colloids that hinder tartrate crystallisation. It was known that the traditional practice of ageing wine on yeast lees (old dead yeast and yeast residues) gave improved tartrate stability. Recent research has established that mannoproteins present in the yeast cell walls are responsible for the improved tartrate stability. This research work underpinned the development of the material which is the subject of this Application[1].

Laffort Oenologie, being the French parent company of the Applicant, holds international patents for a preparation of mannoproteins they have called Mannostab™, which they claim is unique with no equivalent product on the market.

1.3 Function of yeast mannoproteins

Mannoproteins are extracted from purified yeast (*S. cerevisiae*) cell walls, via enzymatic extraction using β-glucosidase exo-1,3 EC [3.2.1.58]. The mannoprotein preparation under consideration in this Application has an apparent molecular weight of around 40 kDa. The function of this mannoprotein preparation is claimed by the Applicant to inhibit tartrate crystallisation in the wine bottle. The Application states that it is proposed to treat wine with yeast mannoproteins in the range of 100-300 mg/L (the maximum proposed treatment level being 300 mg/L).

Mannoproteins of a lower molecular weight (around 32 kDa) have also been found to stabilise wine, however, in this case it is in respect to protein instability (rather than bitartrate inhibition). The protein stabilisation function can potentially reduce or eliminate the requirement to treat wine with a filtration agent, bentonite, which is commonly used to remove excess protein from the wine, which can cause haze instability in the final wine.

Yeast mannoproteins can be called ‘protective colloids’ (another example is gum arabic, also called acacia gum which is currently approved in the Code as a food additive to treat wine).

[1] Ribéreau-Gayon, P., Glories, Y., Maujean, A. and Dubourdieu, D. (2000) Handbook of Enology, Volume 2, The chemistry of wine stabilization and treatments, John Wiley & Sons, pp 15-39.

The Application states that the mechanism for how yeast mannoproteins perform the inhibition of bitartrate crystallisation has been postulated but has not been fully elucidated. It is believed that the mannoproteins (and other protective colloids) adsorb onto the surface of the developing crystal, or crystal nucleation site being protected, to maintain a separation zone around the site and hinder access to approaching molecules or particles, and so limit the growth of the crystal.

1.4 Preparation of yeast mannoproteins

The Application states that the mannoprotein preparation is produced by the β-glucosidase exo-1,3 enzymatic extraction of *S. cerevisiae* yeast cell walls. The β-glucosidase exo-1,3 enzyme preparation is approved for use as a food processing aid; it is listed in the Table to clause 17 of Standard 1.3.3 – Processing Aids of the Code. The enzyme hydrolyses the yeast cell wall which then allows the mannoproteins to be solubilised. Subsequently the enzyme digestion is ultrafiltered to remove insoluble cell wall material and the mannoprotein preparation concentrated.

1.5 International Standards

Yeast mannoproteins are approved for treatment of wine in a number of countries and by the The Organisation Internationale de la Vigne et du Vin (OIV) (International Organisation of Vine and Wine).

The European Union Council Regulation (EC) No. 2165/2005, which amends Regulation (EC) No. 1493/1999 permits 'the addition of yeast mannoproteins to ensure the tartaric and protein stabilisation of wines'[2]. The Commission Regulation (EC) No. 1410/2003 permits the use of preparations of yeast cell walls to the level of 40 g/hl (400 mg/L) for wine[3].

Argentina has approved the use of yeast mannoproteins for wine stabilisation.

The OIV International Oenological Codex (OIV Codex) is an internationally accepted reference for wine related specifications. The OIV Codex includes a specification for yeast mannoproteins in OIV Resolution Oeno 26/2004[4]. This specification indicates that yeast mannoproteins can be used for tartaric and/or protein stabilisation of wine.

The Agreement between Australia and the European Community on Trade in Wine, and Protocol (1994) (Annex I) allows for the use of preparations of yeast cell wall, up to 40 g/hl for wines originating in Australia and separately for wines originating in the Community[5].

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[2] Official Journal of the European Union, Council Regulation (EC) No. 2165/2005 (20 December 2005) http://eur-lex.europa.eu/LexUriServ/site/en/oj/2005/l_345/l_34520051228en00010004.pdf Accessed on 27 July 2007

[3] Official Journal of the European Union, Commission Regulation (EC) No. 1410/2003 (7 August 2003) http://eur-lex.europa.eu/LexUriServ/site/en/oj/2003/l_201/l_20120030808en00090011.pdf Accessed on 27 July 2007

[4] OIV Resolution Oeno 26/2004, Paris (30 July 2004) Yeast mannoproteins, http://news.reseau-concept.net/images/oiv_uk/Client/Resolution_OENO_EN_2004_26.pdf. Accessed on 27 July 2007

[5] Agreement between Australia and the European Community on Trade in Wine, and Protocol (1994), http://www.austlii.edu.au/au/other/dfat/treaties/1994/6.html. Accessed on 27 July 2007

2. The Issue / Problem

There is currently no approval in the Code for the use of yeast mannoproteins to stabilise wine. A pre-market assessment is required before approval for use of food additives in food manufacture is granted. Therefore, a safety assessment is required to assess whether there are any public health and safety issues associated with approving the use of yeast mannoproteins to stabilise wine. FSANZ is also required to assess whether the proposed use is technologically justified and supported.

3. Objectives

The objective of the assessment is to determine whether it is appropriate to amend the Code to permit the use of yeast mannoproteins as a food additive to stabilise wine.

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 *Food Standards Australia New Zealand Act 1991* (FSANZ Act). These are:

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

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

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

4. Key Assessment Questions

The primary role of FSANZ in developing or varying food regulatory measures for food additives is to ensure that the food additive, any potential impurities and the intended level of use do not present public health and safety concerns.

The key questions which FSANZ considered as part of this assessment were:

- Are there any public health and safety issues associated with approving yeast mannoproteins as a food additive to stabilise wine?

- Is there a technological need to use yeast mannoproteins in wine?

FSANZ evaluates public health and safety considerations associated with foods by reviewing available scientific information to estimate, either quantitatively or qualitatively, the probability of an adverse health effect as a result of human exposure to a hazard. In assessing the public health and safety implications of food additives, FSANZ conducts a safety assessment to identify potential public health and safety risks associated with the use of the food additive in the manufacture of food. FSANZ will not approve food additives for inclusion in the Code where a risk to public health and safety is identified.

RISK ASSESSMENT

5. Risk Assessment Summary

5.1 Safety Assessment

A safety assessment was conducted to identify any potential public health and safety risks associated with the use of yeast mannoproteins as a food additive in winemaking. The assessment was based on data on the chemistry, production process, and intended use of the yeast mannoprotein preparation provided by the Applicant and obtained from the scientific literature. FSANZ concluded that there are no safety concerns based on the considerations listed below.

- There is a long history of human consumption of the yeast *S. cerevisiae*, primarily due to its use in baking and brewing.
- Yeast and yeast extracts are safely consumed as dietary and nutritional supplements by humans and animals.
- Mannoproteins released from wine yeast during fermentation are naturally present in wine.
- Yeast mannoproteins are digested as normal dietary proteins.
- The β-glucosidase exo-1,3 enzyme preparation used to extract yeast mannoproteins is an approved food processing aid.
- Product specifications indicate the yeast mannoprotein preparation does not contain chemical or microbiological contaminants above relevant limits.

From the available information, it is concluded that use of this mannoprotein preparation as a food additive in wine would not raise any public health and safety concerns. Further information is provided in the Safety Assessment Report at **Attachment 2**.

5.2 Food Technology Considerations

Yeast mannoproteins extracted from *S. cerevisiae* using enzyme extraction technologies exhibit a technological function in treated wine to either inhibit potassium bitartrate crystallisation or to protein stabilise wine (that is reduce the formation of haze).

This function is as a food additive rather than a processing aid since the mannoproteins act as a stabiliser in the final wine.

The yeast mannoproteins are extracted from the yeast using an approved enzyme, β-glucosidase exo-1,3, (listed in the Table to clause 17 of Standard 1.3.3 – Processing Aids of the Code) which assists in solubilising the mannoproteins from the yeast cell wall material. Further information is provided in the Food Technology Report at **Attachment 3**.

5.3 Dietary Exposure or Nutritional Considerations

FSANZ has estimated the approximate dietary exposure to yeast mannoproteins in wine and foods for Australian and New Zealand populations. The dietary exposure estimate was based on Australian and New Zealand survey data and derived using FSANZ developed dietary modelling software. FSANZ also reviewed dietary exposure data provided by the Applicant.

The Applicant estimated exposures to mannoproteins from food alone, based on French consumption data for adults assuming the French population were a high bread and wine consuming population, and therefore a high yeast and mannoprotein consuming one. From food alone with added mannoproteins in wine, dietary exposure would be 1.66 g mannoproteins per person per day. With added exposure from medical treatments this could be 1.7 g per person per day.

FSANZ estimated dietary exposures for Australian and New Zealand populations from food and beverages, from both naturally occurring and mannoproteins added as proposed in the Application, to be around 0.42 and 0.35 g per person per day, respectively.

The estimated dietary exposures to yeast mannoproteins for high consumers of wine are not significantly higher than population estimates of exposures from all foods. For high consumers of wine in Australia and New Zealand, FSANZ estimated dietary exposures from added sources of mannoproteins only were around 0.3 g per wine consumer per day. This is roughly half the estimated dietary exposures to yeast mannoproteins for Australia and New Zealand from food and beverages from both naturally occurring and added mannoproteins. This was estimated to be around 0.74 and 0.66 g per person per day respectively for high wine consumers. Further information is provided in the Dietary Exposure Assessment Report at **Attachment 4**.

5.4 Risk Characterisation

Based on the conclusions of the safety assessment and exposure assessment, there are no public health and safety concerns associated with the proposed amendment to allow addition of yeast mannoproteins to wine at a maximum proposed concentration of 300 mg/L.

There is a long history of safe human consumption of yeast mannoproteins, primarily through the use of *S. cerevisiae* in baking and brewing, and product specifications indicate the yeast mannoprotein preparation does not contain chemical or microbiological contaminants above relevant limits.

RISK MANAGEMENT

6. Options

FSANZ is required to consider the impact of various regulatory (and non-regulatory) options on all sections of the community, especially relevant stakeholders who may be affected by this Application.

Food additives used in Australia and New Zealand are required to be listed in Standard 1.3.1 – Food Additives. Additives permitted for use in wine production in Australia are listed in Standard 4.5.1 - Wine Production Requirements (Australia only). Yeast mannoproteins are considered to function as a food additive when used to stabilise wine, and require a pre-market approval under Standard 1.3.1. It is not appropriate to consider non-regulatory options.

Two regulatory options have been identified for this Application:

Option 1 Not permit the use of yeast mannoproteins as a food additive to stabilise wine.

Option 2 Amend Standards 1.3.1, 1.3.4 and 4.5.1 to approve the use of yeast mannoproteins as a food additive to stabilise wine.

If the proposed use of yeast mannoproteins is approved, an amendment to Standard 1.3.4 – Identity and Purity would also be required to recognise the OIV Codex or to incorporate a specification as none of the primary or secondary sources listed in clauses 2 and 3 of the Standard apply.

Two options were consulted on at Initial Assessment, recognising the OIV Codex as a whole under clause 3 - Substances with specifications in secondary sources, or including a particular specification for yeast mannoproteins in the Schedule to the Standard. The OIV Codex contains a specification for yeast mannoproteins in OIV Resolution Oeno 26/2004. The OIV Codex is an internationally accepted reference for wine related specifications. To address the specification of yeast mannoproteins, adding the OIV Codex as a secondary source in clause 3 of Standard 1.3.4 is recommended. Referencing a recognised published source containing the relevant specification is preferred to drafting one. Submitters and the Winemakers' Federation of Australia supported referencing the OIV Codex in the Code.

7. Impact Analysis

The impact analysis represents likely impacts based on available information. The impact analysis is designed to assist in the process of identifying the affected parties, any alternative options consistent with the objective of the proposed permission for yeast mannoproteins, and the potential impacts of regulatory provisions. Information from public submissions is sought to make a Final Assessment of the proposed variations to the Code.

7.1 Affected Parties

The parties likely to be affected by the proposed amendments to the Code include:

- the wine industry;

- manufacturers and suppliers of alternative wine stabilisation technologies;
- consumers; and
- Government agencies in Australia and New Zealand involved in enforcing the Code.

7.2 Benefit Cost Analysis

In developing food regulatory measures for adoption in Australia and New Zealand, FSANZ is required to consider the impact of each option on all sectors of the community, including consumers, the food industry and governments. The regulatory impact assessment identifies and evaluates, though is not limited to, the costs and benefits of the regulation, and its health, economic and social impacts. At Final Assessment FSANZ will use the Office of Best Practice Regulation Business Cost Calculator to calculate the compliance costs of regulatory options where medium to significant competitive impacts or compliance costs are likely.

7.2.1 Option 1 – Not permit the use yeast mannoproteins as a food additive to stabilise wine

There are no perceived benefits to industry, consumers or government regulators if this option is progressed.

This option may result in costs to the wine industry in that use of mannoproteins as a food additive that may improve process efficiencies will not be permitted. This may result in a competitive disadvantage for the Australian and New Zealand wine industries internationally as use of the additive is currently permitted in Europe and Argentina and approval in the United States is anticipated.

This option may result in costs to manufacturers and suppliers of alternative wine stabilisation technologies as there will not be an opportunity to market yeast mannoproteins preparations to the wine industry in Australia or New Zealand.

7.2.2 Option 2 – Amend Standards 1.3.1, 1.3.4 and 4.5.1 to approve the use of yeast mannoproteins as a food additive to stabilise wine

For the wine industry, this option would permit the use of an alternative method to stabilise wine, which may result in a more economic and energy efficient production process. The Applicant notes that currently removal of potassium bitartrate is a common, time-consuming and expensive process in winemaking, an additive that would remove this requirement represents a significant advantage in terms of production efficiency, logistics and expenditure. The results of studies conducted by the Applicant indicate that the yeast mannoproteins preparation is effective in preventing potassium bitartrate precipitation when used at the proposed level. The Applicant notes that standard technologies to stabilise wine are also effective but that these methods are less efficient and have higher infrastructure costs and environmental impact, for example, costs associated with refrigeration (cold stabilisation including the contact process) or are not effective in the longer term (metatartaric acid treatment) in comparison to using yeast mannoproteins. No trade implications are anticipated.

Consumers may benefit through the improved appearance and stability of wine. The purpose of the yeast mannoproteins preparation is to prevent potassium bitartrate precipitation in wine.

Wine bitartrate stabilisation is an integral part of the wine production process. If a wine is not stabilised, crystals resembling glass shards are produced in bottled wine. The Applicant notes that while cost savings are anticipated for the wine industry if use of yeast mannoproteins is approved, whether there would be any reduction of the price paid for wine by consumers is a matter for wine producers.

No additional costs to consumers have been identified. The FSANZ Safety Assessment Report **(Attachment 2)** and consideration of dietary implications found no health or safety concerns associated with the use of this mannoprotein preparation as a food additive in wine. FSANZ does not consider there to be any dietary exposure or nutritional implications associated with the proposed approval. As use of yeast mannoproteins has no sensory implications and does not alter the physiochemical characteristics of wine, it is not expected to influence the amount of wine consumed. No increase in the cost of wine to consumers is anticipated, no price increases associated with the use of yeast mannoproteins were observed in the European market following the introduction of the additive there.

No significant additional costs to Government agencies have been identified. Issues raised by one jurisdiction are addressed below in section 9.1.4.

7.3 Comparison of Options

In assessing applications, FSANZ considers the impact of various options on all sectors of the community, including consumers, food industries and governments in Australia and New Zealand. For Application A605, there are no options other than variations to Standards 1.3.1, 1.3.4 and 4.5.1.

Amendments to Standards 1.3.1 and 4.5.1 are required to approve the use of yeast mannoproteins as a food additive to stabilise wine. In addition, an amendment to Standard 1.3.4 is also required to recognise the OIV Codex or incorporate a specification for yeast mannoproteins, as none of the primary or secondary sources listed in clauses 2 and 3 of that Standard apply. Therefore, **option 2** is preferred for the following reasons:

- There are no public health and safety concerns associated with the proposed amendments (this benefit also applies to option 1).

- Use of the additive is technologically justified as an alternative treatment to the currently permitted and used food additives and processes.

- No issues were raised in submissions to the Initial Assessment identifying any risks associated with the proposed approval of yeast mannoproteins to stabilise wine.

The conclusion of the impact analysis is that the benefits of permitting the use of yeast mannoproteins outweigh any associated costs.

COMMUNICATION AND CONSULTATION STRATEGY

8. Communication

As this Application is considered routine and applications to permit food additives do not normally generate public interest, FSANZ has adopted a basic communication strategy, with a focus on informing the community that a change to the Code is being contemplated.

FSANZ publishes the details of the Application and subsequent assessment reports on its website, notifies the community of the public consultation through newspaper advertisements, and issues media releases drawing attention to proposed Code amendments. Once the Code has been amended, FSANZ incorporates the changes in the website version of the Code and, through its email and telephone advice service, responds to industry enquiries.

Should the media show an interest in this Application, FSANZ can provide background information and other advice, as required.

9. Consultation

FSANZ invited public submissions on the Initial Assessment Report. The public comment period commenced on 23 May 2007 and closed six weeks later on 4 July 2007. Seven submissions were received; one opposed and six supported or tentatively supported the Application pending the outcome of the safety assessment. Submissions received during the first round of public comment are summarised in **Attachment 5**.

FSANZ is seeking public comment on this Draft Assessment Report to assist in finalising the assessment of the Application. Comments on, but not limited, to the following would be useful:

- any impacts (costs/benefits) of the proposed variations to Standards 1.3.1, 1.3.4 or 4.5.1;
- any public health and safety considerations associated with the proposed approval; and
- any other affected parties to this Application.

9.1 Issues raised in submissions

9.1.2 Expanding the scope of the Application to include alternative production technologies

DSM Food Specialties suggested in its submission that the scope of the Application should encompass filtration technologies for producing mannoproteins. The company has developed a yeast mannoprotein product using a different production method and states that this product is compliant with the OIV resolution on yeast mannoproteins.

9.1.2.1 FSANZ Evaluation

If this Application is successful and the OIV Codex is included in the Code as a secondary source for specifications, then the OIV specification would be used in determining product compliance with the Code. This would allow scope for other manufacturers to supply yeast mannoprotein products meeting the Standard for use in Australia and New Zealand.

9.1.3 Size of yeast mannoproteins and the OIV specification

DSM Food Specialties notes that the molecular weight of yeast mannoproteins is discussed in the Assessment of the Application and that no reference is made to the size of mannoproteins in the relevant OIV resolution. The company notes that the OIV resolution relates to the stabilising effect and not the size of the mannoproteins and that therefore there would be no requirement in relation to the size of mannoproteins.

9.1.3.1 FSANZ Evaluation

The OIV yeast mannoproteins resolution does not contain a size limit on the molecular weight of the mannoprotein fraction, and states that the yeast mannoproteins can stabilise for tartrate and/or protein in wine. If the Application is successful and the OIV Codex is included in the Code, then this would allow scope for the use of yeast mannoprotein products not only for tartrate stabilisation, but also for protein stabilisation to reduce haze in wine.

9.1.4 Monitoring and compliance costs to Government

One jurisdiction noted that approval of the Application may mean increased monitoring requirements and generate the need to develop an analytical method to police the limit. The submission noted that there could be considerable difficulties involved in attempting to distinguish added mannoproteins from other naturally present proteins in wine. Another jurisdiction did not envisage any significant costs arising from the proposed approval.

9.1.4.1 FSANZ Evaluation

The Application notes that a High Performance Liquid Chromatography (HPLC) analytical method is available to determine the concentration of yeast mannoproteins in wine. As the limit in the Code will apply to the quantity of yeast mannoproteins present in the final product, there would be no need to distinguish between added mannoproteins and other mannoproteins from a compliance perspective.

FSANZ notes that while there may be some costs to governments in relation to monitoring and compliance activities, the impact analysis conducted as part of the assessment of the Application concluded that the benefits of approving the use of yeast mannoproteins to stabilise wine outweigh the costs.

9.1.5 Safety of yeast mannoproteins in relation to vulnerable populations

A member of the public raised concern that mannoproteins may be harmful to diabetics and promote kidney dysfunction. The submitter questions whether wines containing mannoproteins would have warning labels.

9.1.5.1 FSANZ Evaluation

Yeast mannoproteins occur naturally in wine and many other foods. The proposed approval will not be introducing yeast mannoproteins to products which do not already contain them. The Safety Assessment Report and consideration of dietary exposure issues did not identify any health or safety concerns in relation to the use of yeast mannoproteins at the proposed level in wine. The submitter did not provide any evidence or data to justify the claims that mannoproteins may be harmful and FSANZ has not otherwise identified any supporting evidence. The strain of yeast in question has a long and safe history of use. The proposed approval raises no dietary or nutritional concerns and is not considered to raise new issues for diabetics. FSANZ does not consider warning labelling appropriate.

9.1.6 Specification for yeast mannoproteins

At Initial Assessment, consultation was conducted on whether to recognise the OIV Codex as a whole under clause 3 - Substances with specifications in secondary sources, or include a particular specification for yeast mannoproteins in the Schedule to the Standard. No issues or concerns were raised in relation to referring to the OIV Codex in the Code. The Winemakers' Federation of Australia and submitters who commented on this issue all support adding the OIV Codex as a secondary specification source.

9.1.6.1 FSANZ Evaluation

FSANZ recommends referring to the OIV Codex as a secondary source of specifications in Standard 1.3.4 under clause 3. This requires consequential drafting to include 'or' at the end of the previous entry (see **Attachment 1**).

9.2 World Trade Organization (WTO)

As members of the World Trade Organization (WTO), Australia and New Zealand are obligated to notify WTO member nations where proposed mandatory regulatory measures are inconsistent with any existing or imminent international standards and the proposed measure may have a significant effect on trade. Application A605 requests a permission in the Code for the use of yeast mannoproteins to stabilise wine. The proposed regulatory measure is consistent with other international regulation including the OIV International Oenological Codex, European Community regulations and the Agreement between Australia and the European Community on Trade in Wine. Amending the Code to allow the use of yeast mannoproteins as a food additive to stabilise wine is considered unlikely to have a significant effect on trade between member nations. For these reasons it was determined that there is no need to notify this Application as a Sanitary and Phytosanitary (SPS) measure in accordance with the WTO Agreement on the Application of SPS Measures.

CONCLUSION

10. Conclusion and Preferred Approach

This Application has been assessed against the requirements for Draft Assessments in the FSANZ Act. FSANZ recommends the proposed draft variations to Standards 1.3.1, 1.3.4 and 4.5. The draft variations are at **Attachment 1**.

Preferred Approach

FSANZ recommends the proposed draft variations to Standards 1.3.1, 1.3.4 and 4.5.1 to approve the use of yeast mannoproteins as a food additive for wine stability treatment and to recognise the Organisation Internationale de la Vigne et du Vin (OIV) International Oenological Codex (Edition 2006) as a specification source.

10.1 Reasons for Preferred Approach

FSANZ recommends the proposed draft variations to Standards 1.3.1, 1.3.4 and 4.5.1 for the following reasons.

- A detailed safety assessment did not identify any public health and safety concerns.
- Use of the additive is technologically justified as an alternative treatment to the currently permitted and used additives and processes.
- No issues were raised in submissions to the Initial Assessment identifying any risks associated with the proposed approval of yeast mannoproteins.
- The impact analysis concluded that the benefits of permitting the use of yeast mannoproteins as a food additive outweigh any associated costs.
- The proposed variations are consistent with the FSANZ Act section 18 objectives.

11. Implementation and Review

If the variations to the Code proposed through this Application are progressed, the amendments take effect on gazettal and would be subject to existing compliance arrangements.

ATTACHMENTS

1. Draft variations to the *Australia New Zealand Food Standards Code*
2. Safety Assessment Report
3. Food Technology Report
4. Dietary Exposure Assessment Report
5. Summary of Submissions

Attachment 1

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

Section 94 of the FSANZ Act provides that standards or variations to standards are legislative instruments, but are not subject to disallowance or sunsetting

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 –

-	Yeast mannoproteins	300	mg/kg

[2] ***Standard 1.3.4*** *of the Australia New Zealand Food Standards Code is varied by omitting paragraph 3(j), substituting –*

(j) The Japanese Standard for Food Additives 6th Edition (1994); or
(k) Organisation Internationale de la Vigne et du Vin (OIV) International Oenological Codex (Edition 2006).

[3] ***Standard 4.5.1*** *of the Australia New Zealand Food Standards Code is varied by inserting in the* Table to clause 3 –

Table to clause 3

Additive
Yeast mannoproteins

Attachment 2

Safety Assessment Report

1. Introduction

The purpose of this assessment is to determine the safety of a purified yeast (*Saccharomyces cerevisiae*) cell wall preparation consisting of low molecular weight mannoproteins as a food additive in wine. Mannoproteins are highly glycosylated proteins, often 50 to 95% carbohydrate by weight, where the polysaccharide portion is mainly composed of mannose. Mannoproteins inhibit the crystallisation of potassium bitartrate, a salt that is present in wine in a super-saturated concentration. Wine bitartrate stabilisation is often required since, although the presence of potassium bitartrate crystals is not a safety issue, it leads to consumer rejection. The two existing methods, cold stabilisation or metatartaric acid addition, are either costly and inefficient, or have a limited duration of effectiveness.

It is known that macromolecules that are naturally released from yeast during the fermentation process are able to reduce tartrate crystallisation and that barrel-aging white wines on yeast lees for several months often provides sufficient tartrate stability to overcome the need for further stabilisation. The macromolecules responsible for inhibiting crystal formation have been shown to be glycosylated proteins of the yeast cell wall released by enzymatic degradation of the yeast cell wall (Llauberes et al, 1987; Lubbers et al, 1993; Dupin et al, 2000). Addition of a purified yeast cell wall preparation exploits the ability of these mannoproteins to act as protective colloids. While the exact mechanism of this process is unknown, protective colloids are thought to act by coating the site of crystallisation or aggregation and hindering access to nearby particles.

The yeast mannoprotein preparation is added to the wine after fining, just before the final stage of filtration prior to bottling. The product is intended to be used in the range of 100 to 300 mg/L and the Applicant is requesting permission for the use of Mannostab™ at a maximum concentration of 300 mg/L. The correct dose is determined by preliminary testing of each wine, with the recommended dosage being the lowest concentration at which no crystallisation appears plus 50 mg/L. Addition of excess mannoprotein can reduce the stabilising effect.

1.1 Chemistry of yeast cell walls

In *S. cerevisiae*, the cell wall makes up 15 to 30% of the dry weight of the cell and consists of an inner layer of load-bearing polysaccharides, acting as a scaffold for a protective outer layer of mannoproteins (reviewed in Lipke and Ovalle, 1998; Klis et al, 2006). A fibrous β 1,3 glucan-chitin complex is the major constituent of the inner wall. On the outer surface of the wall are mannoproteins, which are highly glycosylated polypeptides. Branched β 1,6 glucan links the components of the inner and outer walls (Table 1). These components are covalently linked to form macromolecular complexes, which are assembled to form the intact wall. Wall components are associated laterally by noncovalent interactions in the glucan-chitin layer and by covalent cross-links in the mannoprotein layer, including disulfide bonds between mannoproteins.

Table 1: Macromolecules[1] of the cell wall of *S. cerevisiae* (from Klis et al, 2006)

Macromolecule	% of wall mass	Mean *M*r (DP[2]) (kDa)
Mannoproteins[3]	30–50	Highly variable
1,6-*β*-Glucan	5–10	24 (150)
1,3-*β*-Glucan	30–45	240 (1500)
Chitin	1.5–6	25 (120)

[1] The cell wall components are presented in the order in which they are found in the cell wall from the outside to the inside.
[2] DP, degree of polymerization (the number of monomer units in an average polymer chain).
[3] The actual protein content is about 4–5%; the remaining mass is from protein-linked, mannose-containing carbohydrate side-chains.

The external protein layer of the cell wall may at any time consist of at least 20 different glycoproteins and the composition of this protein layer may vary depending on growth conditions (Klis et al, 2006). Mannoproteins have different structures depending on their molecular weights and the degree and type of glycosylation. Their molecular weight ranges from 20 kDa to more than 450 kDa.

Many mannoproteins carry N-linked glycans with a core structure of Man10–14GlcNAc2-Asn. Outer chains present on many yeast N-glycans consist of 50 to 200 additional α-linked mannose units, with a long α-1,6-linked backbone decorated with short α-1,2- and α-1,3-linked side chains. There are often several large N-glycans per glycopeptide, so that N-linked sugar can add 50 to 100 kDa to the size of the mannoproteins. Phosphorylation of the mannosyl side chains gives yeast its anionic surface charge.

The mannoproteins can be liberated from the wall by β 1,3 glucanases or β 1,6 glucanases (Lipke and Ovalle, 1998).

2. Production of yeast mannoproteins

2.1 Extraction of yeast mannoproteins

Treatment of yeast with the glucan 1,3-β-glucosidase enzyme hydrolyses the cell wall and allows the mannoproteins to be solubilised. This process is shown diagrammatically in Figure 1. The Applicant states that this enzymatic digestion mimics the natural yeast lysis during fermentation or digestion, during which mannoproteins are released and subsequently absorbed by humans.

The *S. cerevisiae* cell walls, all media and devices used in the yeast mannoprotein production process are of food grade. The *S. cerevisiae* hydrolysis conditions are monitored through the enzymatic process. Each batch of yeast mannoproteins is sterilised by ultrafiltration and analysed to certify the levels of chemical and microbiological contaminants. The mannoprotein concentrate is commercialised in solid form. The product has a long lifespan, being stable for a minimum of two years if kept sealed in a dry location at 12°C.

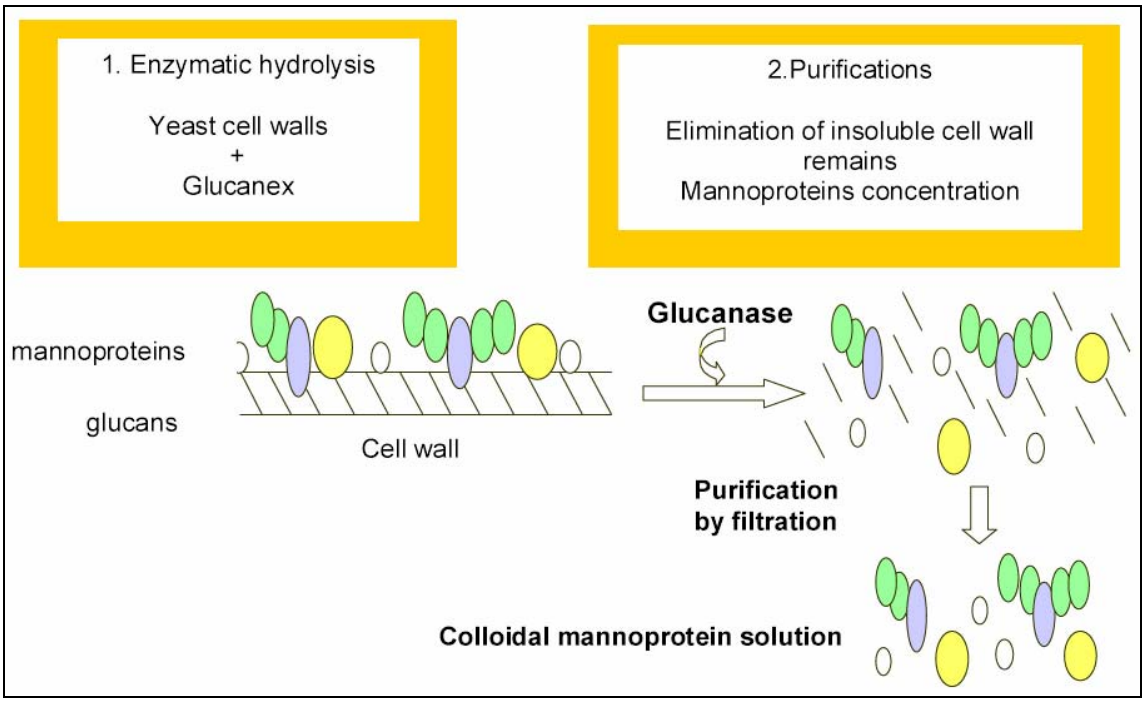

Figure 1: Process for the extraction of mannoproteins from S. cerevisiae.

2.2 Chemical and physical properties of yeast mannoprotein product

The yeast mannoprotein preparation produced by the Applicant is known as Mannostab™. Mannostab™ is biologically derived and is not a single chemical. Mannoproteins have different structures, depending on their molecular weight and degree and type of glycosylation. The molecular weight range of the extracted mannoproteins is 30 - 40 kDa.

Table 2: Chemical and physical properties of yeast mannoproteins

Colour	White or beige
Melting range	Decomposes upon heating
Odour	Nil
Oxidation stability	Stable for two years in a sealed container < 12 °C
Photolysis	Stable
Physical state	Powder
Solubility in organic solvents	Insoluble in ethanol
Solubility in water	Soluble
Thermal stability	Decomposes on excessive heating. Storage to be 4 – 12 °C

2.3 Glucanase enzyme preparation

Yeast mannoproteins are extracted from the cell walls of standard wine yeast (S. *cerevisiae*) by enzymatic treatment of the yeast cell walls with a glucan 1,3-β-glucosidase [3.2.1.58] sourced from the micro-organism *Trichoderma harzianum.* Glucan 1,3-β-glucosidase, also known as exo-1,3-β-glucanase, catalyses the successive hydrolysis of β-D-glucose units from the non-reducing ends of 1,3-β-D-glucans, releasing α-glucose. This enzyme is specified as a permitted food processing aid in the Table to clause 17 of Standard 1.3.3 – Processing Aids of the Code.

The glucan 1,3-β-glucosidase preparation used is known as Glucanex® 200 (Novozymes). Glucanex® is quality assured before sale, including the determination of heavy metal and microbiological contamination levels.

3. Biochemical data

3.1 Digestion of yeast mannoproteins

The yeast mannoprotein preparation is primarily glycans comprised of α-linked mannose units. The preparation contains more than 60 g/100 g polysaccharides (as mannose), approximately 2-4 g/100g carbohydrate, 7-8 g/100g protein, 0.5 g/100g fat and low amounts of minerals and vitamins.

Studies supplied by the Applicant indicate that the yeast mannoproteins are digested like other dietary proteins (Adrian et al, 1996; Moine-Ledoux, 2003). Yeast and yeast cell wall preparations can be used to supplement animal nutrition and have been shown to be an effective protein source and dietary supplement with no reported adverse effects (for example, Spark et al, 2005; Zhang et al, 2005; Merrill et al, 2007; Owens and McCracken, 2007). In the European Community, the Annex to Council Directive 82/471/EEC authorises the use of *S. cerevisiae* cultures or lysates as nutritional substances for all animal species with no restrictions on amounts. Yeast is also used as a nutritional supplement for humans, particularly popular with vegans, as it is regarded as an excellent source of protein and vitamins, especially the B-complex vitamins.

4. Toxicological evaluation

S. cerevisiae is considered to be non-toxic and non-pathogenic. The ability of the yeast species *S. cerevisiae* to ferment sugars has been exploited in baking and fermenting alcoholic beverages for thousands of years, although the direct role of yeast in these processes was not demonstrated until the mid-1800s by Louis Pasteur. Humans are exposed to yeasts and yeast cell walls through use in bakery products, brewed products and in food supplements and human medicines. Wine already contains yeast mannoproteins, released during fermentation (Llauberes et al, 1987; Dupin et al, 2000).

Because of the long history of safe human and animal consumption of yeast, no direct toxicological studies have been reported.

5. Specifications for yeast mannoproteins

The main toxicological consideration of the yeast mannoprotein preparation is in relation to possible contaminants. The specifications to which the Mannostab^{TM} preparation conforms are shown in Table 3.

Table 3: Selected specification limits for yeast mannoproteins (Mannostab™)

Parameter	OIV specification
Assay	> 600 g/kg as mannose
Ash	< 8 %
Appearance	White or beige powder; odourless.
Solubility	Soluble in water; insoluble in ethanol.
Optical rotation	$[\alpha]_D^{20}$ = 80-150º (c = 0.01 g/mL; l= dm)
Moisture content	< 4 %
Preparation of solution for trials	Prepare a 10 g/L solution in water
Heavy metals (other than lead)	< 30 mg/kg
Lead	< 5 mg/kg
Mercury	< 0.15 mg/kg
Arsenic	< 1 mg/kg
Cadmium	< 0.5 mg/kg
Total nitrogen	5 – 75 g/kg
Total aerobic mesophile flora	< 10,000/g
Coliforms	< 10 CFU/g
Staphylococcus aureus	None in a 1 g sample
Salmonella	None in a 25 g sample
Escherichia coli	None in a 25 g sample
Lactic bacteria	< 10^4 CFU/g in a 25 g sample
Mould	< 50 CFU/g
Yeasts	< 10^2 CFU/g

6. Overall Conclusion

From the safety assessment of yeast mannoproteins it has been concluded that:

- there is a long history of human consumption of the yeast S. cerevisiae, primarily due to its use in baking and brewing;
- yeast and yeast extracts are safely consumed as dietary and nutritional supplements by humans and animals;
- mannoproteins released from wine yeast during fermentation are naturally present in wine;
- yeast mannoproteins are digested as normal dietary proteins;
- the exo-1,3-β-glucanase preparation used to extract yeast mannoproteins is an approved food processing aid; and
- product specifications indicate the yeast mannoprotein preparation does not contain chemical or microbiological contaminants above relevant limits.

References

Adrian J, Frangne R, Potus J (1996) les parois de levures alimentaires et leurs incidences nutritionnelles (Shell of food yeasts and their nutritional impacts).*Med. Et Nutr.,* **37**: 167-170.

Dupin IV, McKinnon BM, Ryan C, Boulay M, Markides AJ, Jones GP, Williams PJ, Waters EJ (2000) Saccharomyces cerevisiae mannoproteins that protect wine from protein haze: their release during fermentation and lees contact and a proposal for their mechanism of action. *J Agric Food Chem.* **48**: 3098-3105.

Klis FM, Boorsma A, De Groot PW (2006) Cell wall construction in Saccharomyces cerevisiae. *Yeast*. **23**: 185-202.

Lipke PN, Ovalle R (1998) Cell wall architecture in yeast: new structure and new challenges. *J Bacteriol.* **180**: 3735-3740.

Llauberes RM, Dubourdieu D, Villettaz JC (1987) Exocellular Polysaccharides from *Saccharomyces* in Wine. *J Agric Food Chem.* **41**: 277-286.

Lubbers S, Leger B, Charpentier C, Feuillat M (1993) Essai colloides protecteurs d'extraits de parois de levures sur la stabilité tartrique d'un vin modêle. *J. Inter. Sci Vigne Vin*. **27**: 13-22.

Merrill ML, Bohnert DW, Harmon DL, Craig AM, Schrick FN (2007) The ability of a yeast-derived cell wall preparation to minimize toxic effects of high ergot-alkaloid tall fescue straw in beef cattle. *J Anim Sci.* Jun 25; [Epub ahead of print]

Moine-Ledoux V. (2003) Etude de la digestibilité par les enzymes pancréatiques des levures, de leur paroi et d'une preparation de mannoprotéines, le MannostabTM. Laffort Oenologie report.

Owens B, McCracken KJ (2007) A comparison of the effects of different yeast products and antibiotic on broiler performance. *Br Poult Sci.* **48**: 49-54.

Spark M, Paschertz H, Kamphues J (2005) Yeast (different sources and levels) as protein source in diets of reared piglets: effects on protein digestibility and N-metabolism. *J Anim Physiol Anim Nutr (Berl).* **89**: 184-188.

Zhang AW, Lee BD, Lee SK, Lee KW, An GH, Song KB, Lee CH. (2005) Effects of yeast (Saccharomyces cerevisiae) cell components on growth performance, meat quality, and ileal mucosa development of broiler chicks. *Poult Sci* **84**: 1015-1021.

Attachment 3

Food Technology Report

A605 – Yeast Mannoproteins as a Food Additive for Wine

Summary

Yeast mannoproteins extracted from *Saccharomyces cerevisiae* using enzyme extraction technologies exhibit a technological function in treated wine to either inhibit potassium bitartrate crystallisation or to protein stabilise wine (that is reduce the formation of haze). This function is as a food additive rather than a processing aid since the mannoproteins act as a stabiliser in the final wine.

The yeast mannoproteins are extracted from the yeast using an approved enzyme, β-glucosidase exo-1,3, EC 3.2.1.58 (listed in the Table to clause 17 of Standard 1.3.3 – Processing Aids of the *Australia New Zealand Food Standards Code* (the Code)) which assists in solubilising the mannoproteins from the yeast cell wall material.

The yeast mannoproteins have a specification listed in the Organisation Internationale de la Vigne et du Vin (OIV) (International Organisation of Vine and Wine) International Oenological Codex (OIV Codex).

Introduction

Application A605 from Laffort Services seeks permission to use mannoproteins extracted from yeast cell walls as a food additive in wine to inhibit the crystallisation of potassium bitartrate.

What are yeast mannoproteins?

Yeast mannoproteins are extracted from yeast (*S. cerevisiae*) cell walls, via enzymatic extraction using the enzyme β-glucosidase exo-1,3, EC 3.2.1.58 as listed in the Table to clause 17 of Standard 1.3.3 – Processing Aids of the Code. The accepted IUBMB name for this enzyme is glucan 1,3-β-glucosidase. The enzyme is called Glucanex by the enzyme company Novozymes, and it is sourced from the *Trichoderma harzianum* micro-organism, which is an approved source of the enzyme in the Code.

The yeast *S. cerevisiae* cell walls are mainly composed of mannoproteins and β-1,3 glucans. The outer surface of the cell walls are mainly composed of mannoproteins, which are extensively glycosylated on the O and N groups. Mannoproteins are a number of mannose sugar units linked together by α-links, with a long α-1,6 linked backbone decorated with short α-1,2- and α-1,3 linked side chains (Lipke and Ovalle, 1998).

Technological function in wine

Wine, in particular red wine, naturally contains macromolecules, often called protective colloids that perform a stabilising effect on the wine by hindering tartrate crystals forming in the wine.

Wine is naturally supersaturated with potassium bitartrate (abbreviated often as tartrate) which can crystallise out in the final bottled wine leading to a quality defect, though it is not considered a taste or safety concern. Wine industry practices are employed to address tartrate crystallisation in the aged wine. The most common strategy is cold stabilisation (which involves extended cold storage of the fermented wine to force the crystallisation of tartrate which is removed before the wine is bottled) or the use of metatartaric acid (INS 353) which is a permitted food additive for wine treatment, but it provides only relatively short term stability.

Recently it was discovered that the protective colloids responsible for inhibiting tartrate crystallisation were obtained by enzymatic extraction of mannoproteins from yeast cell walls (Ribéreau-Gayon *et al*. 2000). This research formed the basis of this Application.

It was found that the mannoproteins fraction extracted from yeast cell walls with an average molecular weight of approximately 40 kDa had an inhibiting effect on tartrate crystallisation in wine. Trials have indicated that levels of the extracted yeast mannoproteins between 150 and 250 mg/L inhibit tartrate crystallisation, depending on the wine type.

The mechanism for how the yeast mannoproteins prevent tartrate crystallisation has not been fully elucidated. It is believed that the mannoproteins adsorb onto the surface of the developing crystal or crystal nucleation site, so protecting it by providing a separation zone around the site and hindering access to other tartrate crystals limiting the growth of the crystals.

A separate fraction of yeast mannoproteins recovered from the enzyme treatment of yeast cell walls with an average molecular weight of 31.8 kDa has been shown to provide protein stability to the treated wine. This means that less bentonite (an approved filtration material commonly used in the wine industry to adsorb haze forming protein from the wine) is needed to make the final wine protein stable so preventing haze forming in the aged wine. Research showed that treatment of wine with this yeast mannoprotein fraction (called MP32) at 250 mg/L was shown in research to provide protein stabilisation (Ribéreau-Gayon *et al*. 2000).

This Application seeks approval for yeast mannoproteins to treat wine to inhibit tartrate crystal formation. A slightly different yeast mannoproteins fraction also provides protein stabilisation when added to wine. Both these stabilisation uses are permitted to be used for wine treatment in the European Union (EU) and Organisation Internationale de la Vigne et du Vin (OIV) (International Organisation of Vine and Wine).

Uses of both these forms of yeast mannoproteins would be consistent with use as food additives and not as processing aids since the mannoproteins are contained in the final wine and continue to perform technological functions in the final wine, acting as stabilisers. It is expected that the yeast mannoproteins would be added to the wine after fermentation has been completed, possibly before final filtration to stabilise the wines before they are bottled.

Manufacture of yeast mannoproteins

The enzyme (β-glucosidase exo-1,3, EC 3.2.1.58 being the trade name enzyme Glucanex from Novozymes) hydrolyses the yeast cell wall which then allows the mannoproteins to be solubilised.

The enzyme digestion is filtered to remove insoluble cell wall material and the mannoprotein preparation is concentrated to produce either a colourless, odourless powder or a yellow translucent colloidal solution.

Specifications

The yeast mannoproteins of this Application have a specification listed in the OIV Codex, being resolution OENO 26/2004. This specification does not contain a size limit on the molecular weight of the mannoprotein fraction, and states that the yeast mannoproteins can stabilise for tartrate and/or protein in wine.

Conclusion

Yeast mannoproteins extracted from *S. cerevisiae* using enzyme extraction have been found to have a technological function in treated wine to either inhibit potassium bitartrate crystallisation or to protein stabilise wine (that is reduce the formation of haze). The technological function is as a food additive rather than a processing aid since the mannoproteins act as stabilisers in the final wine.

References

International Union of Biochemistry and Molecular Biology (IUBMB) Enzyme Nomenclature http://www.chem.qmul.ac.uk/iubmb/enzyme/EC3/2/1/58.html, accessed on 3 August 2007.

Lipke, P.N. and Ovalle, R. (1998) Cell wall architecture in yeast: new structure and new challenges. *J. Bacteriol.* **180**: 3735-3740.

OIV Resolution Oeno 26/2004, Paris (30 July 2004) Yeast mannoproteins, http://news.reseau-concept.net/images/oiv_uk/Client/Resolution_OENO_EN_2004_26.pdf. Accessed on 6 August 2007.

Product sheet for Glunanex 200 G, Novozymes http://laffort.com/en/downloads/F-com/Glucanex%20English.pdf access on 3 August 2007

Ribéreau-Gayon, P., Glories, Y., Maujean, A. and Dubourdieu, D. (2000) *Handbook of Enology*, *Volume 2, The chemistry of wine stabilization and treatments*, John Wiley & Sons, pp 15-39 and pp 124-127.

Attachment 4

Dietary Exposure Assessment Report

A605 – Yeast Mannoproteins as a Food Additive in Wine

1. Summary

FSANZ is assessing a request to add yeast mannoproteins to wine as a food additive to inhibit the crystallisation of potassium bitartrate. Mannoproteins are found naturally in foods, primarily foods containing yeast.

FSANZ reviewed some dietary exposure data provided by the Applicant. FSANZ also estimated dietary exposures for yeast mannoproteins for Australian and New Zealand populations aged 18 years and above.

The Applicant estimated exposures to mannoproteins from food and beverages, based on French consumption data for adults assuming the French population were a high bread and wine consuming population, and therefore a high yeast and mannoprotein consuming one. Assuming added mannoproteins in wine and naturally occurring mannoproteins in other foods, mean dietary exposure would be 1.66 g mannoproteins per person per day. With added exposure from medical treatments, the mean exposure could be 1.72 g mannoproteins per person per day.

Mean dietary exposures from food and beverages, from both naturally occurring and added sources as proposed in the Application, was estimated by FSANZ to be lower than that for the French population, around 0.42 g per person per day for the Australian population and 0.35 g per person per day for the New Zealand population. For high consumers of wine, estimated dietary exposures to mannoproteins from wine and foods were 0.74 g per day for Australian adults and 0.66 g per day for New Zealand adults.

2. Background

An Application was received by Food Standards Australia New Zealand (FSANZ) requesting permission to add yeast mannoproteins as a food additive to wine to inhibit the crystallisation of potassium bitartrate. Yeast mannoproteins are proposed to be added to wine in the range of 100-300 mg/L.

3. Evaluation of the dietary implications, as provided by the Applicant

The Applicant identified three main routes of human exposure to the yeast *S. cerevisiae*:

1. yeasts used in bakery, breakfast cereals, brewery and vinification;
2. dietary supplements; and
3. medicines.

The exposure from supplements was identified by the Applicant as not being able to be estimated. The exposure from medicines can be up to 360 mg of *Saccharomyces cerevisiae* per day for 3 months of chronic treatment.

Data from the Bakery Yeast Manufacturers Committee of the European Union indicated that yeast in bakery products is 2-5% (2-5 kg yeast/100 kg flour).

The Applicant noted the naturally occurring levels of mannoproteins in foods as:

- Beer – mean 192 mg/L (range 83-507 mg/L);
- Wine – 125 mg/L; and
- Bread/pastries – maximum 8.5 g/kg[6].

The Applicant provided estimates of dietary exposure based on the French population who were assumed to be high level consumers of bread and wine and therefore of yeast and mannoproteins. The mean consumption amounts for foods containing yeasts, provided from a French consumption survey, were included for adults and children.

The Applicant provided 2 scenarios of dietary exposure based on the French consumption data, assuming mannoproteins were added to wine. Estimates were made for adults only based on the assumption that children do not consume wine.

Scenario 1 was an estimate of dietary exposure to mannoproteins from:

- Foods (breads etc) at 1.6 g/person/day;
- natural occurrence in beer and wine, 0.014 g/person per day and 0.005 g/person per day respectively; and
- mannoproteins added to wine (at 300 mg/L), 0.033 g/person per day.

The total estimated dietary exposures to mannoproteins were 1.66 g/person per day, with 2% of dietary exposure coming from added sources of mannoproteins.

Scenario 2 was an estimate of dietary exposure to mannoproteins from food/beverages and medical treatment. The total exposure from food (Scenario 1) of 1.66 grams mannoproteins per day was added to exposure from medical treatment (0.06 g mannoproteins/day) to get a total of 1.72 grams mannoproteins per person per day.

4. Estimated dietary exposure for Australians and New Zealanders

In order to determine whether estimated exposures to mannoproteins would be in the same range for Australia and New Zealand as predicated by the Applicant, dietary exposures for adults aged 18 years and above (in order to capture alcohol consuming adults) were estimated. Two different assessments were conducted: the first estimating dietary exposure from all foods and beverages from natural and added sources of mannoproteins for the population, and the second for high consumers of wine.

4.1 Estimated mean dietary exposure to mannoproteins from foods and wine

Firstly, the amount of yeast consumed from bread and other yeast-containing products, such as breads and bread based dishes, doughnuts, pastries and crumbed products etc was estimated.

[6] If bread contains 5% yeast (50 g yeast per kg bread), then taking into account that 50% of the yeast is cell wall and that 34% of the cell wall is mannoprotein, the maximum mannoprotein content of bread is 8.5 g/kg bread.

This was based on the 1995 Australian National Nutrition Survey (NNS) and the 1997 New Zealand NNS food consumption data and the recipes constructed for mixed foods (e.g. breads, crumbed fish etc.) in DIAMOND[7]. The dietary exposure estimate to mannoproteins from yeast in these products (see Table 1) was then based on information provided in the Application that 50% of the yeast cell is cell wall and 34% of the cell wall is released as mannoproteins:

Amount of mannoproteins (g/day) = Amount of yeast (g/day) x 50% x 34%

The dietary exposure estimate for mannoproteins from yeast extract spreads was based on the assumption that the spreads were 100% yeast and used the formula outlined above, deriving food consumption amounts for yeast spreads from the 1995 and 1997 NNSs (Table 2).

The exposure to mannoproteins from beer and wine for the population (all respondents) aged 18 years and above was estimated using the mannoprotein concentration supplied by the Applicant and mean consumption amounts for all adult respondents from the 1995 and 1997 NNSs.

The estimated mean dietary exposures to mannoproteins from all added and naturally occurring sources was around 0.42 grams per person per day for Australian adults aged 18 years and above and around 0.35 grams per person per day for New Zealanders aged 18 years and above (see Table 3).

The estimated dietary exposures to mannoproteins from food and wine for Australia and New Zealand were lower than those estimated by the Applicant based on food consumption data from France. This can be explained by several factors. The Applicant assumed around 9 grams yeast per person per day from breads and pastries (assuming 5% of breads and pastries are yeast). The consumption of yeast from breads and bread based dishes, doughnuts, pastries and crumbed products etc. was based on recipes in DIAMOND and was around 1 gram per day for Australia and New Zealand. The consumption of yeast from yeast extract spreads was around 0.9 grams per day for Australia and New Zealand. There was no yeast extract spread consumption included in the exposure estimates provided by the Applicant. The amount of wine consumption used by the Applicant was around double that for Australia and New Zealand.

4.2 Estimated dietary exposure to mannoproteins for high wine consumers

The 95th percentile consumption of wine for respondents aged 18 years and above was combined with the maximum proposed concentration of mannoproteins in the wine of 425 mg/L (300 mg/L added, natural level of 125 mg/L). The estimated dietary exposures to mannoproteins from wine (added and natural) are shown in Table 4. Estimated dietary exposures to mannoproteins from the high consumption (95th percentile) of wine only were 0.34 grams per day for Australian adults and 0.32 grams per day for New Zealand adults.

If it was assumed that high wine consumers have average exposures to mannoproteins from all foods other than wine, the estimated dietary exposures to mannoproteins for high wine consumers were 0.74 grams per day for Australian adults and 0.66 grams per day for New Zealand adults.

[7] DIAMOND is FSANZ's computer program that is used to conduct dietary exposure assessments.

These estimated dietary exposures to mannoproteins for high consumers of wine were higher than the population estimates of mean dietary exposures to mannoproteins from all foods (0.42 g/day for Australia; 0.35 g/day for New Zealand).

Table 1: Consumption of yeast and amount of mannoproteins consumed from bread and similar products for Australians and New Zealanders aged 18 years and above

Country	Number of respondents[#]	Mean consumption yeast for all respondents[#] (g/day)	Amount of mannoproteins (g/day)[^]	Number of consumers[*]	Mean consumption yeast for consumers[*] only (g/day)	Amount of mannoproteins (g/day)[^]
Australia	10,986	1.3	0.22	9,526	1.5	0.26
New Zealand	4,449	0.9	0.15	3,965	1.0	0.17

A respondent is any person included in the NNS, irrespective of whether they consumed wine or not.
* A consumer is a respondent from the NNS who consumes wine.
^ Calculated using the amount of yeast consumed and assuming 50% of the yeast is cell wall and 34% of the cell wall is released as mannoproteins (e.g. 1.3 g yeast x 0.5 x 0.34 = 0.22 g mannoproteins per day).

Table 2: Consumption of yeast and amount of mannoproteins consumed from yeast extract spreads for Australians and New Zealanders aged 18 years and above

Country	Number of respondents[#]	Mean consumption yeast for all respondents[#] (g/day)	Amount of mannoproteins (g/day)[^]	Number of consumers[*]	Mean consumption yeast for consumers[*] only (g/day)	Amount of mannoproteins (g/day)[^]
Australia	10,986	0.84	0.14	1,843	5.03	0.86
New Zealand	4,449	0.94	0.16	733	5.69	0.97

A respondent is any person included in the NNS, irrespective of whether they consumed wine or not.
* A consumer is a respondent from the NNS who consumes wine.
^ Calculated by amount of yeast consumed and assuming that yeast extract spreads are 100% yeast, 50% of the yeast is cell wall and 34% of the cell wall is released as mannoproteins (e.g. 0.84 g yeast x 0.5 x 0.34 = 0.14 g mannoproteins per day).

Table 3: Estimated mean dietary exposures to mannoproteins from all food sources for Australian and New Zealand adults aged 18 years and above

Food	Estimated respondent mean dietary exposure to mannoproteins (g/day)	
	Australia	**New Zealand**
Bakery products	0.22	0.15
Yeast extract spreads	0.14	0.16
Beer	0.04	0.03
Wine (naturally occurring plus added)	0.02	0.01
Total	**0.42**	**0.35**

Table 4: Estimated dietary exposure to mannoproteins from high consumption of wine for Australians and New Zealanders aged 18 years and above

Country	Number of consumers of wine	Wine consumption (L/day)		Concentration of mannoproteins (mg/L)	Estimated exposure to mannoproteins from wine only (g/day)	
		Mean respondents[#]	95th percentile consumers[*]		Mean for respondents[#]	95th percentile[*]
Australia	1,555	0.05	0.8	425	0.02	0.34
New Zealand	495	0.03	0.75	425	0.01	0.32

Number of respondents 18 years and above = Australia 10,986; New Zealand 4,449.

A respondent is any person included in the NNS, irrespective of whether they consumed wine or not.

* A consumer is a respondent from the NNS who consumes wine.

Attachment 5

Summary of Submissions

Round one – Initial Assessment

At Initial Assessment early input was sought on a range of specific issues known to be of interest to various stakeholders in relation to the Application.

Public comment was sought on the following issues:

- the safety of the use of mannoproteins to treat wine;
- whether use of yeast mannoproteins to treat wine would cause any deleterious effects to wine;
- food technology issues arising from the use of yeast mannoproteins to treat wine;
- applicable international regulation in general and the appropriateness of including the OIV International Oenological Codex as a secondary source for specifications in the Code in particular; and
- cost benefit impacts.

Submissions were received from DSM Food Specialties, the Food Technology Association of Australia Inc., the Queensland Government, the New Zealand Food Safety Authority, the Australian Food and Grocery Council (AFGC), the NSW Food Authority and Paul Elwell-Sutton. Comments are summarised in the table below.

Submitter	Comments
DSM Food Specialties	Supports the Application and including the OIV Codex in the Code noting that this would add to the consistency between Australian and international standards. DSM has developed a yeast mannoprotein product using a different production method and states that this product is compliant with the OIV resolution on yeast mannoproteins. Suggests that the scope of A605 should encompass filtration technologies for producing mannoproteins. Notes that the molecular weight of yeast mannoproteins is discussed in the Assessment and that no reference is made to the size of mannoproteins in the OIV resolution. Notes that the OIV resolution relates to the stabilising effect and not the size of the mannoproteins and that therefore there would be no requirement in relation to the size of mannoproteins.
Food Technology Association of Australia Inc.	Supports option 2 to amend the Code to approve the use of yeast mannoproteins as a food additive for wine to inhibit potassium bitartrate crystallisation.

Submitter	Comments
Queensland Government	Offered tentative support for option 2 to amend the Code to approve the use of yeast mannoproteins as a food additive for wine to inhibit potassium bitartrate crystallisation. Final position reliant upon reviewing documentation supplied by the Applicant and FSANZ particularly as it relates to the safety assessment of the use of yeast mannoproteins to stabilise wine. The submission states that approval of the Application may mean increased monitoring requirements and generate the need to develop an analytical method to police the limit. The submission notes that there could be considerable difficulties involved in attempting to distinguish added mannoproteins from other naturally present proteins in wine.
New Zealand Food Safety Authority	Supports option 2 in principle. May comment further at Draft Assessment.
Australian Food and Grocery Council	Supports option 2 to amend the Code to approve the use of yeast mannoproteins as a food additive in wine, conditional on safety. The AFGC advocates that food additives and processing aids should be permitted providing that they are safe when used at the intended levels. AFGC notes that use of yeast mannoproteins is technologically justified, recognised internationally and approved by the OIV. The AFGC notes that there is a long history of use of the strain of yeast used and that there are no safety concerns with the yeast or the extraction method. The AFGC notes that potassium bitartrate crystallisation commonly occurs in wine bottles and that approval of the additive would enable the wine industry to become more cost competitive against producers in countries where it is already permitted as current practices used to stabilise wine are time consuming and expensive. The AFGC states that approval of the additive will allow wine producers to make a more cost effective product and potentially provide greater competition and reduced prices for consumers.
NSW Food Authority	Supports consideration of the Application and does not envisage any significant costs to the Authority arising from the proposed approval.
Paul Elwell-Sutton	Opposes the Application on the grounds that mannoproteins may be harmful to diabetics and promote kidney dysfunction. The submitter questions whether wines containing mannoproteins would have warning labels and rates FSANZ's performance on labelling as unsatisfactory from a consumer perspective.