



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

04/03

9 October 2002

INITIAL ASSESSMENT REPORT

APPLICATION A475

HEXOSE OXIDASE AS A PROCESSING AID (ENZYME)

DEADLINE FOR PUBLIC SUBMISSIONS to the Authority in relation to this matter:

20 November 2002

(See "Invitation for Public Submissions" for details)

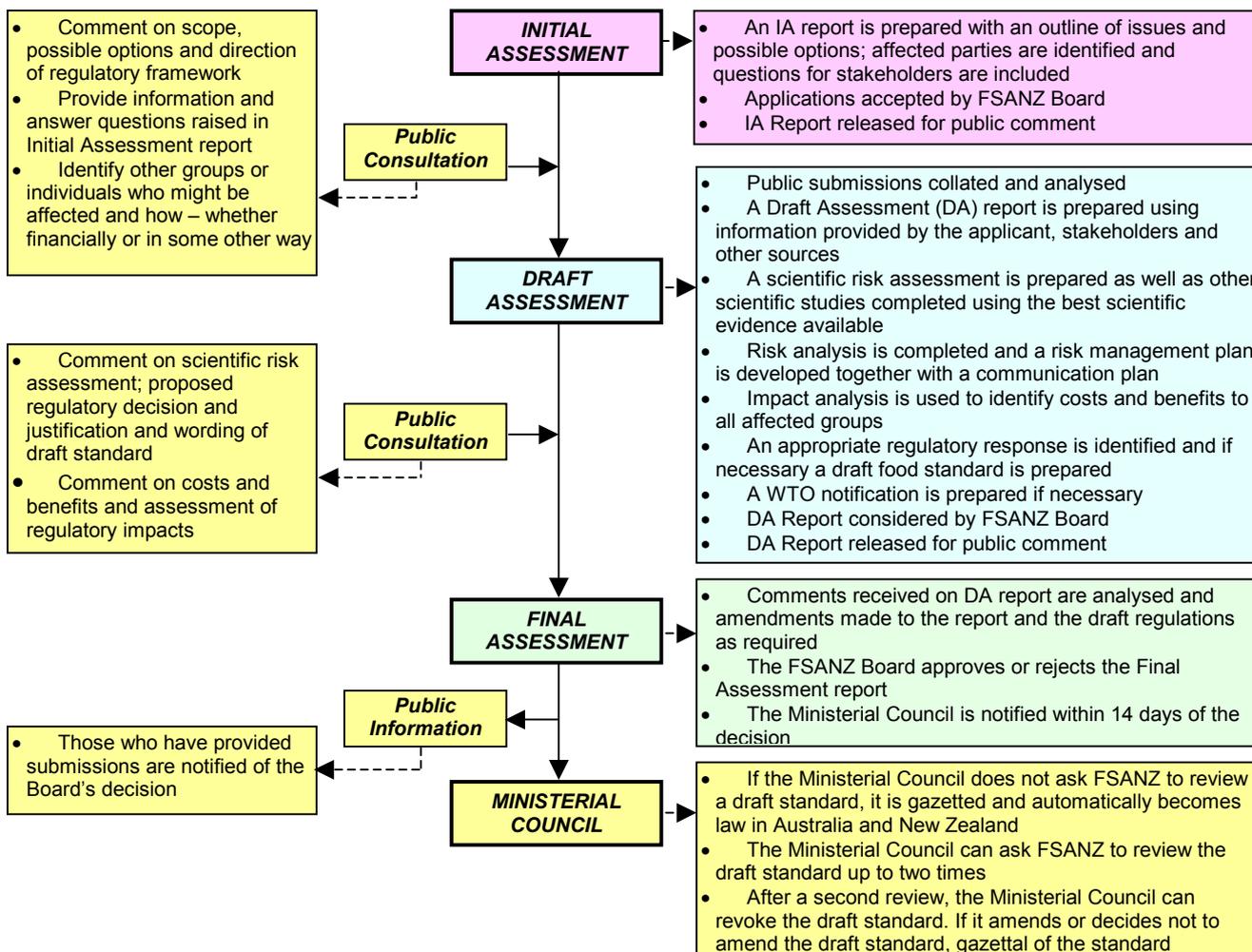
FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

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 *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

The Authority has prepared an Initial Assessment Report of Application A475, which includes the identification and discussion of the key issues.

The Authority invites public comment on this Initial Assessment Report based on regulation impact principles and the draft variation to Volume 2 of the *Food Standards Code* for the purpose of preparing an amendment to the *Food Standards Code* for approval by the FSANZ Board.

Written submissions are invited from interested individuals and organisations to assist the Authority in preparing the Draft Assessment for this application. Submissions should, where possible, address the objectives of the Authority as set out in Section 10 of the *Food Standards Australia New Zealand Act 1991* (FSANZ Act). Information providing details of potential costs and benefits of the proposed change to the *Food Standards Code* (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 the Authority are open to public scrutiny, and any submissions received will ordinarily be placed on the public register of the Authority and made available for inspection. If you wish any information contained in a submission to remain confidential to the Authority, you should clearly identify the sensitive information and provide justification for treating it as commercial-in-confidence. Section 39 of the FSANZ Act requires the Authority 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 the Authority by: **20 November 2002**. Submissions received after this date may not be considered unless the Project Manager has given prior agreement for an extension. Submissions may also be sent 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 or alternatively paper copies of reports can be requested from the Authority’s Information Officer at either of the above addresses or by emailing info@foodstandards.gov.au including other general enquiries and requests for information.

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Executive Summary

An application has been received 8 August 2002, from Danisco A/S to amend Standard 1.3.3 of the *Australia New Zealand Food Standards Code (Food Standards Code)* to approve the use of an enzyme, hexose oxidase as a processing aid. The hexose oxidase is produced, using recombinant DNA techniques, from the host yeast *Hansenula polymorpha* which contains the donor gene coding for hexose oxidase from the algae *Chondrus crispus*.

This Initial Assessment report is not a detailed assessment of the application but rather an assessment that the application should be accepted for further consideration. The report is based mainly on information provided by the applicant and has been written to assist in identifying the affected parties and to outline expected relevant issues to complete the assessment. The information needed to complete the assessment will include information received from public submissions.

There is currently no approval for the use of hexose oxidase as a food enzyme in the Food Standards Code. If this application is successful FSANZ will add permission to use the enzyme hexose oxidase produced by *Hansenula polymorpha*, carrying the gene coding for hexose oxidase isolated from *Chondrus crispus* to the Table to clause 17 of Standard 1.3.3 – Processing Aids.

The objective of this assessment is to determine whether it is appropriate to amend the *Food Standards Code* to permit the use of hexose oxidase from the above source.

Hexose oxidase catalyses the oxidation of various mono and oligosaccharides to lactones and hydrogen peroxide. Its main application is in bread making to increase dough strength and bread volume. It acts in a similar way to glucose oxidase for this purpose however it is claimed to have added advantages. Other applications in the food industries are in cheese and tofu manufacture where it aids curd formation, limiting undesirable browning by limiting Maillard reactions in food and as an oxygen scavenger in dressings and sauces.

The enzyme is isolated from the red algae *Chondrus crispus*. However this source is not a suitable production organism since recoveries are low and not economic. The gene for the enzyme was therefore inserted into the host yeast *Hansenula polymorpha* from which the enzyme can be recovered in economic quantities using a submerged fermentation process.

The applicant claims that the gene, the vector and the host organism are all well characterised. The donor organism, *Chondrus crispus*, has a long history of safe use in food. The host organism, *Hansenula polymorpha* is non-toxicogenic.

The hexose oxidase enzyme preparations comply with the specifications for food enzyme preparations in Food Chemicals Codex, 4th Edition 1996, and also the Joint FAO/WHO Expert Committee on Food Additives (JECFA), in the Compendium of Food Additives Specifications, FAO Food and Nutrition Paper 52, Vol. 1, Annex 1 (1992), including relevant updates in addenda 1 to 9 (2001).

In the USA a GRAS (generally recognised as safe) expert panel has concluded that the enzyme is safe for food use as a processing aid. The enzyme is approved for use in baked goods (at a level up to 150 enzyme units/kg) in Denmark.

1. Introduction

An application has been received 8 August 2002, from Danisco A/S to amend Standard 1.3.3 of the *Australia New Zealand Food Standards Code (Food Standards Code)* to approve the use of an enzyme, hexose oxidase as a processing aid. The hexose oxidase is produced, using recombinant DNA techniques, from the host yeast *Hansenula polymorpha* which contains the donor gene coding for hexose oxidase from the algae *Chondrus crispus*.

Hexose oxidase catalyses the oxidation of various mono and oligosaccharides to lactones and hydrogen peroxide. Its main application is in bread making to increase dough strength and bread volume. It acts in a similar way to glucose oxidase for this purpose however it is claimed to have added advantages where it has wider substrate specificity. Other applications in the food industries are in cheese and tofu manufacture where it aids curd formation, limiting undesirable browning by limiting Maillard reactions in food and as an oxygen scavenger in dressings and sauces.

2. Regulatory Problem

Processing aids are required to undergo a pre-market safety assessment before approval for use. A processing aid is a substance used in the processing of raw materials, foods or ingredients, to fulfil a technological purpose relating to treatment or processing, but does not perform a technological function in the final food.

There is currently no approval for the use of hexose oxidase as a food enzyme in the *Food Standards Code*. Hexose oxidase is not listed in the Table to clause 17 of Standard 1.3.3 – Processing Aids.

The *Australia New Zealand Food Standards Code* will be the sole food regulatory Code on 20 December 2002. FSANZ is therefore only considering an amendment to the *Australia New Zealand Food Standards Code* (known as Volume 2) and will not be considering an amendment to the *Australian Food Standards Code* (known as Volume 1).

3. Objective

The objective of this assessment is to determine whether the *Food Standards Code* should be amended to permit the use of hexose oxidase from the above source. The assessment will need to be consistent with the section 10 objectives of the *Food Standards Australia New Zealand Act 1991*.

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

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

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

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

4. Background

It has been known that hexose oxidase can be extracted from a number of red algae. However due the difficulty in recovering the small amounts of the enzyme from such algae little use has been made of its properties. This has been overcome in recent times by using recombinant DNA technologies to produce greater quantities of enzyme to enable more complete characterisation. Such techniques have allowed industrial processes to be used to produce commercially viable enzyme preparations. The gene encoding for the enzyme has been isolated from the algae *Chondrus crispus* and inserted into the yeast host *Hansenula polymorpha*. The subsequent organism is able to produce the enzyme in commercial quantities during a submerged fermentation process.

5. Relevant Issues

5.1 Nature of the enzyme

The common name of the enzyme is hexose oxidase (HOX) while its chemical name is D-hexose: oxygen 1-oxidoreductase. The Enzyme Commission number is EC 1.1.3.5 and the CAS registry number is 9028-75-5.

The enzyme is produced by fermentation of a selected strain of the yeast *Hansenula polymorpha* modified with the hexose oxidase encoding gene isolated from the algae *Chondrus crispus*.

The enzyme catalyses the oxidation of various mono and oligosaccharides (principally glucose, but also maltose, lactose, D-galactose, D-mannose and cellobiose) with oxygen to produce lactones and hydrogen peroxide. In aqueous solutions the lactones hydrolyse over time to the corresponding acids. The hydrogen peroxide acts as an oxidant with other food components.

5.2 Efficacy and technological justification

Hexose oxidase acts in a comparable way to glucose oxidase, but is claimed to be more effective and can be used in a wider range of products.

The applicant claims the enzyme can be used alone or in combination with other oxidants such as ascorbic acid in bread making where it is added to the dough. The enzyme catalyses the oxidation of thiol groups in the gluten structure forming disulphide bonds so strengthening the gluten network of the dough and improving the dough handling. The baked bread is larger and has better properties. The enzyme can replace other chemical bread improvers such as potassium bromate which is no longer permitted for this purpose in Australia and New Zealand.

The application claims that hexose oxidase can be used in a number of foods to limit unwanted browning reactions (Maillard) that occur with the naturally occurring oligosaccharides, by oxidizing them so they are unable to react. Such a case is shredded cheese (such as Mozzarella used on the top of pizzas) where the lactose and galactose naturally occurring in cheese form strong Maillard reactions during baking so producing strong brown baked colours. The use of the enzyme limits colour generation which may be desirable for certain products.

The applicant claims a similar case of limiting the formation of browning Maillard reactions in potato chip production. Sprouted potatoes have a higher concentration of reducing sugars, and chips produced from such potatoes form undesirable brown colours when fried. Treatment with the enzyme limits the browning.

Hexose oxidase is claimed to also oxidise the reducing sugars so that whiter egg powders and whey powders can be produced due to limiting browning reactions.

The application claims that lactose in skim milk and soya milk can be oxidised by hexose oxidase to form the acid, lactobionic acid, which aids in the precipitation of curd for cottage cheese and tofu manufacture respectively.

The applicant believes the enzyme can be added as an oxygen scavenger during sauce and dressing manufacture reducing dissolved oxygen concentrations in the products which improves the shelf life of resulting products.

The applicant states that they have not marketed their enzyme preparations in Australia or New Zealand so they have not supplied any letters from food manufacturers supporting and justifying a need for the application. Supporting letters will be supplied for the Draft and Final assessment stage.

A Food Technology Report included as part of the Draft Assessment Report will investigate more fully the purpose and efficacy of the enzyme.

5.3 Safety assessment

The applicant claims that the gene, the vector and the host organism are all well characterised. The donor organism, *Chondrus crispus*, has a long history of safe use in food. The host organism, *Hansenula polymorpha* is non-toxicogenic.

The applicant has assessed the safety of the enzyme by providing the following studies:

1. Acute oral toxicity studies in the rat;
2. Ames mutagenicity studies;
3. In vitro chromosomal aberration study;
4. Sub-acute 14-day toxicity study in the rat;
5. Sub-chronic 90-day feeding study in the rat.

These toxicity studies will be assessed as part of a Safety Assessment Report prepared for the Draft Assessment Report.

5.4 Other international regulatory standards

In the USA a GRAS (generally recognised as safe) expert panel has concluded that the enzyme is safe for food use as a processing aid. The formal US Food Drug Administration (FDA) GRAS notification has not yet been made.

The enzyme is approved for use in baked goods (at a level up to 150 enzyme units/kg) in Denmark.

The applicant states that the hexose oxidase enzyme preparations comply with the specifications for food enzyme preparations in Food Chemicals Codex, 4th Edition 1996, and also the Joint FAO/WHO Expert Committee on Food Additives (JECFA), in the Compendium of Food Additives Specifications, FAO Food and Nutrition Paper 52, Vol. 1, Annex 1 (1992), and relevant updates in addenda 1 to 9 (2001).

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 *Food Standards Code* will be analysed using regulatory impact principles.

The following two regulatory options are available for this application:

- Option 1.** Not approve the use of hexose oxidase produced by *Hansenula polymorpha* carrying the gene coding for hexose oxidase isolated from *Chondrus crispus* as a food processing aid.
- Option 2.** Approve the use of hexose oxidase produced by *Hansenula polymorpha* carrying the gene coding for hexose oxidase isolated from *Chondrus crispus* as a food processing aid.

7. Impact Analysis

The affected parties to this application include those listed below:

1. those sectors of the food industry wishing to produce and market food products produced using hexose oxidase as a processing aid;
2. consumers; and
3. State, Territory and New Zealand government enforcement agencies that enforce food regulations.

The impact of the proposed change to the regulation will be determined at the Draft Assessment.

8. Consultation

8.1 Public consultation

FSANZ is seeking public comment in order to assist in assessing this application. There will also be a further round of public comment after the Draft Assessment report is completed.

Comments on the following topics would be useful:

- Technological justification;
- Safety considerations;
- Other scientific aspects; and
- Costs and benefits.

8.2 Workplan Classification

FSANZ's scoping of this application for placement on the Workplan was Group 3, Category 2 (information about the Workplan and the different groups and categories are provided in the document *Information for Applicants* on FSANZ's website at www.foodstandards.gov.au). The initial assessment confirms that this Workplan assignment is appropriate.

8.3 World Trade Organization (WTO)

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

Amending the *Food Standards Code* to approve the enzyme hexose oxidase produced by *Hansenula polymorpha* carrying the gene coding for hexose oxidase isolated from *Chondrus crispus* as a processing aid is unlikely to have a significant effect on trade. The enzyme preparations are also consistent with the international specifications for food enzymes of Food Chemicals Codex (4th Edition, 1996) and JEFCA so there does not appear to be a need to notify the WTO. However this issue will be fully considered at Draft Assessment and, if necessary, notification will be made in accordance with the WTO Technical Barrier to Trade (TBT) or Sanitary and Phytosanitary Measure (SPS) agreements.

9. Conclusion and Recommendation

This application fulfils the requirements for Initial Assessment as prescribed in section 13 of the *Food Standards Australia New Zealand Act 1991* and should be accepted.

Accordingly FSANZ has decided to accept the application and will now proceed to conduct a Draft Assessment and prepare a Draft Assessment Report.