

**06/03**

**18 December 2002**

## **DRAFT ASSESSMENT REPORT**

### **APPLICATION A467**

#### ***ALPHA-AMYLASE AS A PROCESSING AID (ENZYME)***

**DEADLINE FOR PUBLIC SUBMISSIONS** to the Authority in relation to this matter:  
**12 February 2003**

*(See “Invitation for Public Submissions” for details)*

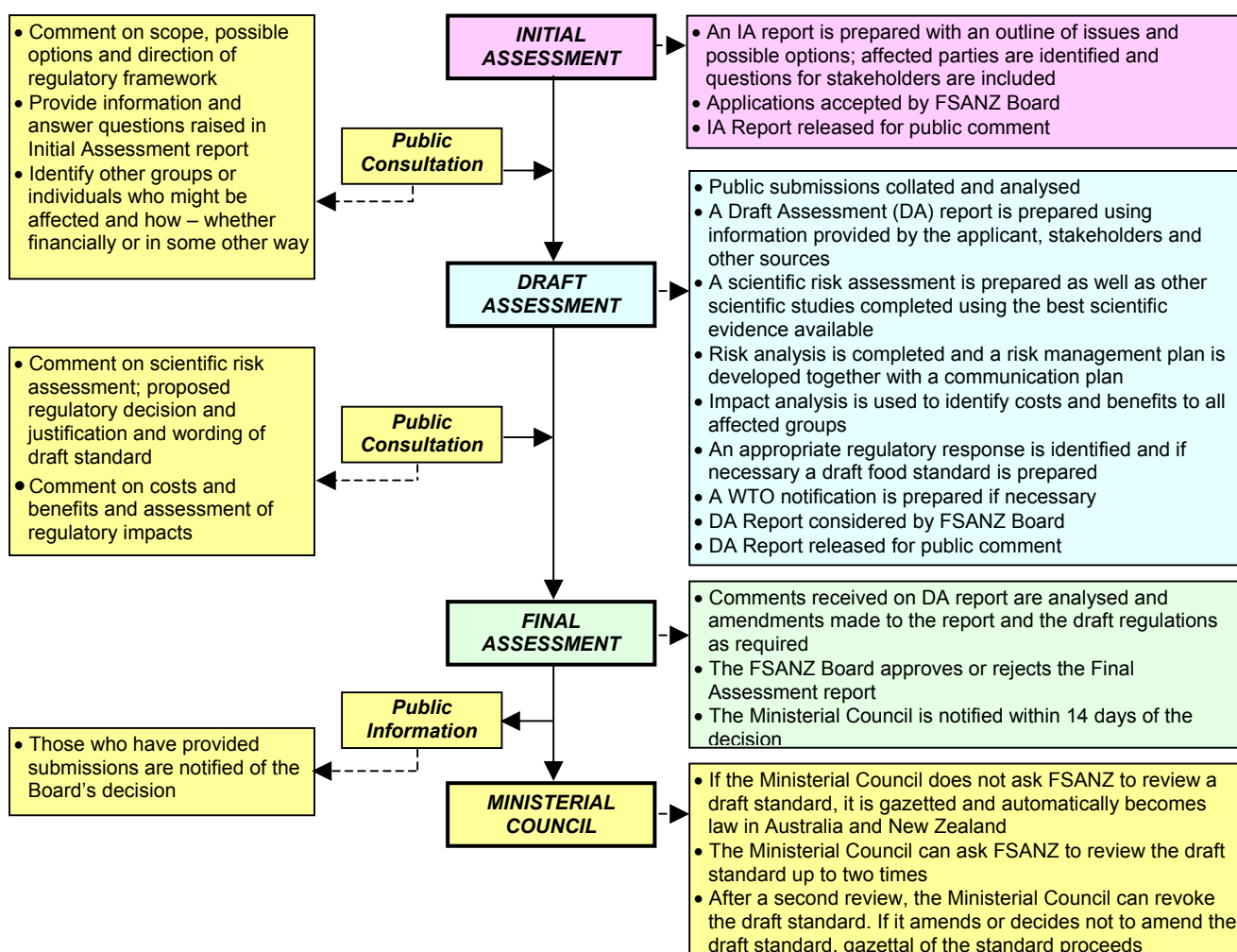
## FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

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

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

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

The process for amending the *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 a Draft Assessment Report of Application A467 and prepared a draft variation to Volume 2 of the *Food Standards Code*.

The Authority invites public comment on this Draft 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 Final 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](http://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](http://www.foodstandards.govt.nz)**

Submissions should be received by the Authority by: **12 February 2003**. 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](mailto: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](mailto:info@foodstandards.gov.au) including other general enquiries and requests for information.

## CONTENTS

<b>EXECUTIVE SUMMARY AND STATEMENT OF REASONS .....</b>	<b>5</b>
STATEMENT OF REASONS.....	5
<b>1. INTRODUCTION.....</b>	<b>7</b>
<b>2. REGULATORY PROBLEM.....</b>	<b>7</b>
<b>3. OBJECTIVE .....</b>	<b>7</b>
<b>4. BACKGROUND .....</b>	<b>8</b>
<b>5. RELEVANT ISSUES .....</b>	<b>8</b>
5.1 NATURE OF THE ENZYME .....	8
5.2 EFFICACY AND TECHNOLOGICAL JUSTIFICATION.....	8
5.3 SAFETY ASSESSMENT .....	9
5.4 OTHER INTERNATIONAL REGULATORY STANDARDS.....	9
<b>6. REGULATORY OPTIONS.....</b>	<b>10</b>
<b>7. IMPACT ANALYSIS .....</b>	<b>10</b>
7.1 OPTION 1.....	10
7.2 OPTION 2.....	10
<b>8. CONSULTATION .....</b>	<b>11</b>
8.1 PUBLIC CONSULTATION.....	11
8.2 WORLD TRADE ORGANIZATION (WTO) .....	11
<b>9. CONCLUSION AND RECOMMENDATION .....</b>	<b>11</b>
<b>ATTACHMENT 1 -DRAFT VARIATIONS TO THE <i>FOOD STANDARDS CODE</i> .....</b>	<b>13</b>
<b>ATTACHMENT 2 - SUMMARY OF PUBLIC SUBMISSIONS.....</b>	<b>14</b>
<b>ATTACHMENT 3 - SAFETY ASSESSMENT REPORT .....</b>	<b>15</b>
<b>ATTACHMENT 4 - FOOD TECHNOLOGY REPORT.....</b>	<b>22</b>

## EXECUTIVE SUMMARY AND STATEMENT OF REASONS

An Application has been received from Genencor International to amend the *Australia New Zealand Food Standards Code* (the Code) to approve the use of an enzyme, *alpha*-amylase derived from *Bacillus stearothermophilus* (brand names G-zyme, G-995 and G-997) as a processing aid. The Application was received on 29 May 2002 and work commenced on 9 July 2002.

*Alpha*-amylase is used as a food enzyme for the hydrolysis of starch in the starch, sugar and alcohol beverage industries. Genencor's *alpha*-amylase is produced with the use of a non-genetically modified strain of *Bacillus stearothermophilus*.

*Alpha*-amylases have been approved and used for many years in food manufacture. There are currently a number of approved *alpha*-amylases listed as processing aids in Standard 1.3.3 of the Code.

If this Application is approved Standard 1.3.3 – Processing Aids in the *Australia New Zealand Food Standards Code* (Volume 2) will be amended. The relevant standard in the *Australian Food Standards Code* (Volume 1) will not be amended because Volume 1 is expected to be repealed in December 2002.

The purpose of this assessment is to determine whether to amend the Code to permit the use of *alpha*-amylase derived from *Bacillus stearothermophilus* as a processing aid.

The only regulatory options considered were to approve or not approve this application. Approval of the use of this enzyme has advantages for food manufacturers by providing a different source of the *alpha*-amylase enzyme; one, which has greater thermal stability and produces a different sugar profile. There are no significant disadvantages to food manufacturers, consumers or government agencies.

Public comment on the Initial Assessment Report for this application was sought from 21 August until 2 October 2002. Three submissions were received. All supported approval of the use of the enzyme – subject to an appropriate safety assessment as part of the Draft Assessment.

FSANZ is now seeking public comment on this Draft Assessment to assist in the Final Assessment. Comments on the following points would be useful:

- Technological justification;
- Safety considerations; and
- Interest from industry in using the enzyme in food manufacture.

### Statement of Reasons

The draft variation to Standard 1.3.3 – Processing Aids of the Code, thereby giving approval for the use of *alpha*-amylase derived from *Bacillus stearothermophilus* as a processing aid is recommended for the following reasons:

- There are no public health and safety concerns associated with the use of the enzyme preparation.

- The use of the *alpha*-amylase enzyme is technologically justified since it has a role in food manufacturing, primarily with starch hydrolysis. The enzyme from *Bacillus stearothermophilus* has greater thermostability, results in a different sugar profile and promotes cost competition to other enzyme suppliers.
- The source organism (*Bacillus stearothermophilus*) has a long history of safe use.
- The *alpha*-amylase enzyme has a history of safe use for many years in Australia and New Zealand.
- The enzyme preparations of *alpha*-amylase derived from *Bacillus stearothermophilus* complies with the specifications in Food Chemicals Codex (4<sup>th</sup> Edition, 1996) and the Compendium of Food Additives Specifications, Vol. 1, Annex 1, FAO 1992, (updated in Addendum 9, 2001).
- The proposed draft variation to the Code is consistent with the section 10 objectives of the FSANZ Act. FSANZ protects public health and safety by assessing the safety of the enzyme preparation to its use in the food supply. The assessment is based on the best available scientific data. It will allow food manufacturers to have a range of enzyme supplies so encouraging an efficient and internationally competitive industry.
- The benefits of using the enzyme for food manufacturers outweigh any costs associated with its use.

## 1. Introduction

An Application has been received on 29 May 2002, from Genencor International to amend the Code to approve the use of an enzyme, *alpha*-amylase derived from *Bacillus stearothermophilus* (brand names G-zyme, G-995 and G-997) as a processing aid.

*Alpha*-amylase is used as a food enzyme for the hydrolysis of starch in the starch, sugar and alcohol beverage industries. Genencor's *alpha*-amylase is produced with the use of a non-genetically modified strain of *Bacillus stearothermophilus*.

## 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 are currently approved sources of *alpha*-amylase listed in Standard 1.3.3 – Processing Aids, of the Code, however *Bacillus stearothermophilus* is not an approved source for *alpha*-amylase so an application to consider varying the Code is required. The Table to clause 17 of Standard 1.3.3 list of approved sources of *alpha*-amylase includes recombinant *Bacillus licheniformis* and *Bacillus subtilis*, both containing the gene for *alpha*-amylase isolated from *Bacillus stearothermophilus*. *Bacillus stearothermophilus*, is not an approved organism as a source for *alpha*-amylase within the Code.

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

## 3. Objective

The objective of this assessment is to determine whether it is appropriate to amend the Code to permit the use of another source of *alpha*-amylase. Such an amendment will need to be consistent with the section 10 objectives of the FSANZ Act.

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

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

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

- the need for standards to be based on risk analysis using the best available scientific evidence;
- the promotion of consistency between domestic and international food standards;
- the desirability of an efficient and internationally competitive food industry;
- the promotion of fair trading in food; and

- any written policy guidelines formulated by the Ministerial Council.

FSANZ is protecting public health and safety by checking that the enzyme is safe for use and that there are no significant health risks associated with approval of the new enzyme source. This report, including the safety assessment, ensures that the best available scientific data has been evaluated for the purposes of conducting a risk assessment. The assessment of this Application will allow manufacturers to have a range of options encouraging an efficient and internationally competitive food industry, plus promoting consistency with other international food standards.

## 4. Background

Enzymatic processes including the use of *alpha*-amylase have been used for several decades in place of acid hydrolysis in industrial processes for starch conversion. They have been used since they offer advantages including greater yields, better control and specificity of products, and improved economics because of milder conditions with lower energy requirements (temperatures and times of reactions).

*Alpha*-amylase degrades both the branched and unbranched forms of starch and related polysaccharides and oligosaccharides by cleaving the internal *alpha*-1,4 bonds connecting the glucose monomers.

*Alpha*-amylase is used in the starch, sugar and alcoholic industries. It is used to liquefy starch to produce soluble dextrans, which can be converted further with other enzymes to produce a range of liquid syrups. Such sugar syrups can then be used in a range of foods. *Alpha*-amylase can also be used in baking to supplement natural sources of the enzyme coming from the grain. The enzyme can also be used in the brewing industry to supplement the natural sources of the enzyme during various steps in beer production and also for different specialty beers (such as low-carbohydrate beers).

## 5. Relevant Issues

### 5.1 Nature of the enzyme

The common name of the enzyme is *alpha*-amylase. The chemical name is 1,4 *alpha*-D-glucan glucanohydrolase with the Enzyme Commission number EC [3.2.1.1] and CAS number 9000-90-2. The marketing names for the commercial enzyme preparations are G-zyme G-995 and G-zyme G-997.

The *alpha*-amylase enzyme catalyses the endohydrolysis of 1-4-*alpha*-D-glucosidic linkages in polysaccharides containing three or more 1,4-*alpha*-linked D-glucose units. The Food Technology report (at Attachment 4) examined the mechanism of action, advantages and production of the *alpha*-amylase from *Bacillus stearothermophilus*.

### 5.2 Efficacy and technological justification

*Alpha*-amylase derived from *Bacillus stearothermophilus* has advantages over other *alpha*-amylases currently approved in the Code. It offers greater thermostability (enzyme activity maintained at higher temperatures) compared to enzymes from *Aspergillus oryzae* and *Bacillus subtilis*. It also produces different sugar profiles to that produced by the enzyme from *Bacillus licheniformis*.



Penford Australia, who produces a range of glucose syrups from wheat starch, sent a letter supporting this application as part of the application. Their letter was to establish a commercial need for the application on the grounds of them needing a high temperature *alpha*-amylase, which is involved in one of the stages of producing glucose syrups and maltodextrins. Penford states that it is an enzyme derived from a non-genetically modified source organism and will provide price competition for enzyme suppliers.

Such sugar syrups and maltodextrin powders are used in a wide variety of food industries including confectionery, dairy foods, ice cream, general foods, beverages and health foods. The enzyme may also be used in the alcoholic beverage industry.

It is concluded that the use of the enzyme is technologically justified (Food Technology Report - Attachment 4)

### 5.3 Safety assessment

*Alpha*-amylases have been used safely as enzyme preparations in food processing for many decades. The safety of *alpha*-amylase derived from *Bacillus stearothermophilus* has been examined (Attachment 3). This safety assessment of the *Bacillus stearothermophilus alpha*-amylase found that:

- The source organism *Bacillus stearothermophilus* has a long history of safe use as a production strain for food-grade enzyme preparations;
- The enzyme preparation complies with the Joint FAO/WHO Expert Committee on Food Additives (JECFA) specifications;
- The enzyme preparation produced no evidence of genotoxic potential in *in vitro* assays;
- There was no evidence of toxic effects of *Bacillus stearothermophilus alpha*-amylase in the acute and sub-chronic dosing studies in animals.

From the information available, it is concluded that the use of *Bacillus stearothermophilus alpha*-amylase as a processing aid in food would not pose public health and safety risk.

There are currently two approved sources of *alpha*-amylase within the Table to clause 17 in Standard 1.3.3 of the Code where the gene for *alpha*-amylase isolated from *Bacillus stearothermophilus* and added into two other hosts (*Bacillus licheniformis* and *Bacillus subtilis*).

As with most enzymes there are not expected to be any dietary considerations since *alpha*-amylase is used as a processing aid in the initial stage of production of sugar syrups. The heating steps inactivate the enzyme and the subsequent purification steps remove most, if not all, of the enzyme (or protein).

### 5.4 Other international regulatory standards

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) at their thirty-seventh session (1990) established an ADI of 'not specified' for *alpha*-amylase from *Bacillus stearothermophilus* as there was no evidence of public health problems.

In 1995, The Food and Drug Administration of the United States (FDA) affirmed the generally recognised as safe (GRAS) status of the *alpha*-amylase derived from *Bacillus stearothermophilus*.

The Applicant states that the *alpha*-amylase enzyme preparations comply with the specifications for food enzyme preparations in Food Chemicals Codex (FCC), 4<sup>th</sup> Edition, 1996, and also the Joint FAO/WHO Expert Committee on Food Additives (JECFA), in the Compendium of Food Additives Specifications, Vol. 1, Annex 1, FAO (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 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 *alpha*-amylase derived from *Bacillus stearothermophilus* as a food processing aid.
- Option 2.** Approve the use of *alpha*-amylase derived from *Bacillus stearothermophilus* 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 *alpha*-amylase as a processing aid;
2. consumers; and
3. State, Territory and New Zealand Government enforcement agencies that enforce food regulations.

### **7.1 Option 1**

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

There are disadvantages to those food industries that wish to use this source of the *alpha*-amylase enzyme. Especially those that wish to use its advantage of higher inactivation temperature and different sugar profile.

### **7.2 Option 2**

There are advantages to food manufacturers to be able to use this different source of *alpha*-amylase enzyme. It is in competition to other approved sources so possibly providing price and supplier competition. This different enzyme source also has advantages to the other approved sources in having greater thermal stability and being able to produce a different sugar profile.

There should be no added costs to government regulators or consumers.

Option 2, which supports the approval of *alpha*-amylase derived from *Bacillus stearothermophilus* as a food processing aid is the preferred option, since it has advantages for the food industry and consumers but has no significant cost for government regulators, consumers or manufacturers.

## **8. Consultation**

### **8.1 Public consultation**

The Initial Assessment Report for this application was circulated for a round of public comment from 21 August till 2 October. Three submissions were received. All supported option 2 – to approve the use of the enzyme – subject to an appropriate safety assessment as part of the Draft Assessment Report.

Attachment 2 summarises the submissions received during the first round of public comment.

FSANZ is now seeking further public comment on this Draft Assessment Report to assist in assessing this application at Final Assessment.

Comments that would be useful could include:

- technological justification;
- safety considerations; and
- other relevant scientific aspects.

### **8.2 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 Code to approve the enzyme *alpha*-amylase derived from *Bacillus stearothermophilus* 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 (4<sup>th</sup> Edition, 1996) and JECFA so FSANZ considers there is no need to notify the WTO.

## **9. Conclusion and Recommendation**

The Draft Assessment Report concludes that approval of the use of the enzyme *alpha*-amylase derived from *Bacillus stearothermophilus* as a food processing aid is technologically justified and does not pose a significant risk to public health and safety.

The draft variation to Standard 1.3.3 – Processing Aids of the Code, thereby giving approval for the use of the enzyme *alpha*-amylase derived from *Bacillus stearothermophilus* as a processing aid is recommended for the following reasons:

- There are no public health and safety concerns associated with the use of the enzyme preparation.

- The use of the *alpha*-amylase enzyme is technologically justified since it has a role in food manufacturing, primarily with starch hydrolysis. The enzyme from *Bacillus stearothermophilus* has greater thermostability, results in a different sugar profile and promotes cost competition to other enzyme suppliers.
- The source organism (*Bacillus stearothermophilus*) has a long history of safe use.
- The *alpha*-amylase enzyme has a history of safe use for many years in Australia and New Zealand.
- The enzyme preparations of *alpha*-amylase derived from *Bacillus stearothermophilus* complies with the specifications in Food Chemicals Codex (4<sup>th</sup> Edition, 1996) and the Compendium of Food Additives Specifications, Vol. 1, Annex 1, FAO 1992, (updated in Addendum 9, 2001).
- The proposed draft variation to the Code is consistent with the section 10 objectives of the FSANZ Act. FSANZ protects public health and safety by assessing the safety of the enzyme preparation to its use in the food supply. The assessment is based on the best available scientific data. It will allow food manufacturers to have a range of enzyme supplies so encouraging an efficient and internationally competitive industry.
- The benefits of using the enzyme for food manufacturers outweigh any costs associated with its use.

## ATTACHMENTS

1. Draft variations to the *Food Standards Code*.
2. Summary of Public Submissions.
3. Safety Assessment Report.
4. Food Technology Report.

**Draft variations to the *Food Standards Code***

**To commence: on gazettal**

[1] *Standard 1.3.3 of Volume 2 of the Food Standards Code is varied by inserting in the Table to clause 17, for the enzyme  $\alpha$ -Amylase EC [3.2.1.1] the Source –*

<i>Bacillus stearothermophilus</i>
------------------------------------

SUMMARY OF PUBLIC SUBMISSIONS

**Round One**

**Submitters**

#	Submitter Organisation	Name
1	Australian Food and Grocery Council	Tony Downer
2	Food Technology Association of Victoria	David Gill
3	Western Australian Food Advisory Committee	Virginia McLaughlin

Submitter	Comments
Australian Food and Grocery Council	<p>Support the application subject to an appropriate safety assessment. Support the statement that <i>Bacillus stearothermophilus</i> is considered non-pathogenic and non-toxigenic. It is the donor organism for a number of currently approved enzymes.</p> <p>They state there are technological reasons why the enzyme use is justified even though there are currently other approved <i>alpha</i>-amylase enzymes. These include:</p> <ul style="list-style-type: none"> <li>• price – the enzyme could be cheaper;</li> <li>• temperature sensitivity – different to other sources, important to have different optimum temperatures ranging through cold to hot;</li> <li>• rapidity of operation – this can vary from slow to fast and is critical to the appropriate processing;</li> <li>• efficiency – the mass of product produced per unit of enzyme can often vary.</li> </ul> <p>States that manufacturers will make a decision on which enzyme preparation to use based on a combination of these factors.</p>
Food Technology Association of Victoria	Support option 2, to approve the use of the enzyme as a food processing aid.
Western Australian Food Advisory Committee	Support option 2, to approve the use of the enzyme. Accept there is substantial evidence that the enzyme is safe but will review the Safety Assessment Report that will be part of the Draft Assessment when it is written.

## SAFETY ASSESSMENT REPORT

### 1. Introduction

Application A467 seeks approval for the use of *alpha*-amylase from a non-genetically modified *Bacillus stearothermophilus* as a processing aid.

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

### 2. The source (production) organism - *Bacillus stearothermophilus*

The safety of the production organism is an important consideration in the safety assessment for enzymes used as a processing aid. *Bacillus stearothermophilus* is considered to be non-pathogenic and nontoxic, and has a long history of safe use as a production strain for food-grade enzyme preparations<sup>1</sup>. In particular this strain and its derivatives have been used for the production of food grade *alpha*-amylase for two decades.

### 3. Purity of enzyme preparation and proposed specifications

Historically, enzymes used in food processing have been found to be non-toxic, and the main toxicological consideration is in relation to possible contaminants. The production organism in this case is non-toxic and non-pathogenic. The detailed specifications to which the preparation was found to conform are shown in Table 1.

**Table 1. Complete specification of alpha amylase preparation**

Criteria	Specification
Heavy Metals	not more than 30 ppm
Potassium sorbate	0.25-0.4 % w/w
Sodium benzoate	1.0- 1.7 % w/w
Sodium chloride	>10%
Arsenic	Not more than 3 ppm
Lead	not more than 5 ppm
Total viable count (cfu/g)	not more than $5 \times 10^4$
Total coliforms (cfu/g)	not more than 30
Production organism (cfu/ml)	<1
Mycotoxins	Negative by test
Antibacterial activity	Negative by test
Total carbohydrate	18-25% w/w
pH	5.5.- 6.0
<i>Salmonella</i>	negative by test

<sup>1</sup> Pariza, M.W. and E.A. Johnson, Evaluating the safety of microbial enzyme preparations used in food processing: update for a new century. *Reg. Toxicol. Pharmacol.* **33**, 173-186 (2001).

*Alpha*-amylase from the source organism, *B. stearothermophilus* complies with the recommended purity specifications for food-grade enzymes<sup>2,3</sup>.

#### 4. Evaluation of the submitted studies

Five toxicological studies were submitted in support of this application. These were: a) acute oral toxicity study in rats, b) a 90-day sub-chronic oral toxicity study in dogs, c) a 90-day sub-chronic oral toxicity study in weanling rats, d) a bacterial mutagenicity assay and e) a human lymphocyte cytogenetic assay. The test material was produced in the same manner as the commercial preparations. The enzyme activity is measured as the amount of enzyme required to hydrolyse 10 mg starch per minute under the conditions of assay. The catalytic activity was measured to be 3900- 6540 U/g with an amount of 10% Total Organic Solids.

##### 4.1 Acute oral toxicity study with *Bacillus stearothermophilus* $\alpha$ -amylase in rats.

*Study number 81213* by G.W. Thompson, F. E. Reno and T.E. Palmer, Hazelton Raltech, Inc. Hazelton Laboratories America, Inc, USA. *March 30, 1982.*

Test material:	<i>Bacillus stearothermophilus</i> $\alpha$ -amylase
Test Species:	Fischer 344 albino rats, 10 males and 10 females per test dose, administration via gavage.
Dose:	Acute doses at 0, 2.5, 5.0 and 10.0 g/kg bw.

##### *Study conduct*

Rats were administered test article via gavage at 0, 2.5, 5.0 and 10.0 g/kg bw/day. They were observed for clinical signs at 1-hour intervals for 8 hours post dosing; and daily for 14 days for any mortality and clinical signs. Rats were provided with rodent diet *ad libitum* except for an overnight fasting period prior to dosing. Body weights were recorded before and after fasting on day 0 and on days 7 and 14 post-dosing. Animals were necropsied on day 14 post-dose.

##### *Results & Conclusion*

There were no deaths, clinical signs, effects on bodyweights or gross necroscopy findings related to treatment.

##### 4.2 Ninety-day subchronic oral toxicity study of *Bacillus stearothermophilus* $\alpha$ -amylase in dogs.

*Study No. 81170* by P.S. Mac and W.N. Hauck. Hazelton Raltech, Inc. Hazelton Laboratories America, Inc, USA. *February 24, 1983.*

Test material:	<i>Bacillus stearothermophilus</i> $\alpha$ -amylase
Test Species:	Beagle dogs 4 males and 4 females per test dose, administration in diet
Dose:	0, 0.56 or 1.11% in diet for 13 weeks.

---

<sup>2</sup> Joint FAO/WHO Expert Committee on Food Additives (JECFA), 2001. General specifications and considerations for enzyme preparations used in food processing. FAO Food and Nutrition Paper 52, Add. 9, pp. 37-39.

<sup>3</sup> National Academy of Sciences, Food and Nutrition Board, Committee on Food Chemical Codex. 1996. *Food Chemical Codex*, 4<sup>th</sup> edition, National Academy Press, Washington DC.



Guidelines: USFDA, non-clinical Laboratory Studies, GLP Regulations  
(21 CFR 58)

### *Test article*

The test material *Bacillus stearothermophilus*  $\alpha$ -amylase, as a lyophilised powder had an activity of 6540 units/g and a total protein value of 71.8%.

### *Study conduct*

Three groups of dogs (4/sex/group) were treated with 0, 0.56, or 1.11% of the test article in the diet equivalent to 0, 36 or 72 units  $\alpha$ -amylase /g. During the acclimation period, all animals were fed the basal diet.

Clinical observations, bodyweight and food consumption were recorded weekly; haematology, clinical chemistry and urinalysis every month; and ophthalmology of all animals was performed before the study and near termination. At the end of the study, all animals were sacrificed and a complete necropsy performed (gross examination and organ weights tissue sampling). Macroscopic and microscopic examination of terminally sacrificed males and females were conducted and histopathological parameters were measured.

### *Results*

No deaths were associated with treatment. There were no treatment related clinical signs, adverse effects on food consumption and body weights or bodyweight gains. The reporting ophthalmologist concluded that there were no ocular abnormalities associated with the test material.

Analysis of the blood chemistry parameters revealed a slight but statistically significant decrease in the direct bilirubin values of the Group 3 males after 1 month but returned to normal afterwards. Total protein and globulin concentrations decreased after the completion of 2 months in the Group 2 and Group 3 female dogs. After the completion of 3 months the total protein concentration in Group 3 males was also reduced ( $p < 0.05$ ) compared to the controls.

Urinalysis showed an increase in the urine specific gravity of Groups 2 and 3 females were increased ( $\leq 0.05$ ).

Organ weights were generally unaffected by treatment up to the highest dose. The absolute weight and relative adrenal weights of the Group 2 females were lower than the control animals ( $\leq 0.01$ ) but the variation is isolated and not considered to be treatment-related.

Macroscopic observations revealed redness on the mucosal surfaces of the caecum, or duodenum or jejunum of some animals. Microscopic examination of these events was unremarkable. Other findings were inflammation and mononuclear cell infiltration of the liver and tube mineralisation in the kidney. These occurrences were isolated and random and therefore were not considered to be treatment-related.

### *Conclusion*

The observed decreases in the total protein and globulin concentrations are not toxicologically significant as the changes were small and the values were within limits of those previously observed for Beagle dogs of similar age and sex.

Further, the changes were not correlated with clinical observations or with differences in body weight and feed consumption data. The dogs were all healthy right through the experiment. In conclusion, no evidence of toxicity was noted following treatment with *Bacillus stearothersophilus*  $\alpha$ -amylase at levels up to 1.11% in the diet.

### 4.3 Subchronic oral toxicity study of *Bacillus stearothersophilus* $\alpha$ -amylase in *in utero* exposed F1 rats.

*Study No. 81168* by J Mielenz & K.M. MacKenzie, Hazelton Raltech, Inc., Hazelton Laboratories America, Inc, USA. *March 18, 1983.*

Test material:	<i>Bacillus stearothersophilus</i> $\alpha$ -amylase
Test Species:	CDF (F-344)/CrIBR rats 12 males and 24 females per P <sub>1</sub> dose group; 20 F <sub>1</sub> animals /sex/group
Dose:	0, 0.56, or 1.11% (w/w) in diet for 13 weeks.
Guidelines:	USFDA, non-clinical Laboratory Studies, GLP Regulations (21 CFR 58)

#### *Test article*

The test material *Bacillus stearothersophilus*  $\alpha$ -amylase, as a lyophilised powder had an activity of 6540 units/g and a total protein value of 71.8%.

#### *Study Conduct*

Groups of 12 male and 24 female Fischer 344 rats were treated with *Bacillus stearothersophilus*  $\alpha$ -amylase in the diet at 0, 0.56, or 1.11% (w/w) (equivalent to 0, 36 or 72 units  $\alpha$ -amylase /g). P<sub>1</sub> animals were treated 13 weeks before mating. The animals were sacrificed after 22 weeks. At 4 weeks of age selected F<sub>1</sub> males and females (20/sex/group) were dosed as above for 13 weeks prior to terminal sacrifice.

A clinical examination was performed twice daily, food consumption weekly (except during pairing) and bodyweights measured weekly (pre-mating, gestation and lactation for females).

Detailed necropsy was performed on adult P<sub>1</sub> and F<sub>1</sub> animals. Histopathology was carried out on all F<sub>1</sub> animals in the control and high dose groups.

#### *Results*

*P<sub>1</sub> generation*-There were no deaths or abnormal or dose related clinical observations during the study that was attributed to treatment. Ophthalmic examination revealed ocular lesions or irritations as the most common occurrences. These were diagnosed to have been caused by non-specific coronal virus. Variations in mean bodyweight were observed only in females during weeks 14 through 22 due to gestation and lactation periods. Mean body weight gains were lower for both sexes treated with 0.56% and 1.11% test article at various time periods. Food consumption was lower in females during weeks 2,4,5,10 and 11; whereas, for males, there were no significant differences in mean food consumption between test and control groups throughout the treatment period.

No significant differences between the mean values of the control groups and the treated groups were observed in the haematology, clinical chemistry or urinalysis carried out after 6 and 12 weeks of treatment.

*F<sub>1</sub> generation*- Clinical observations revealed no abnormalities. All animals appeared healthy till the end of the experiment. There were no differences in mean body weights between test groups and control groups. In general, mean body weight gain was lower for treated animals. The mean food consumption was higher for the treated male rats, but not for female rats, when compared to control group. Only three animals had eye lesions and none were treatment related.

A few minor differences in serum enzyme activities were noted but they were not treatment related. There was no significant difference in haematology or urinalysis values detected between the treated animals and the control animals.

There was no observable difference in the absolute organ weights of *F<sub>1</sub>* rats sacrificed at 13-weeks post-weaning; however relative weights of some organs (spleen, kidneys and brain) showed statistically significant variations. These changes are due to increased terminal body weights of the treated animals accounting for the decreases in the relative weights of kidneys and brain. The changes are not considered biologically important as confirmed by macroscopic and microscopic observations at necropsy.

### *Conclusions*

Administration of *Bacillus stearothermophilus*  $\alpha$ -amylase to *P<sub>1</sub>* rats for 13 weeks followed by post weaning administration of *F<sub>1</sub>* rats for a further 13 weeks did not produce treatment-related effects in the haematology, urinalysis or clinical pathology data. Microscopic and macroscopic examinations did not reveal any abnormalities.

In conclusion daily treatment with test substance at concentrations of up to 1.11% (72 units  $\alpha$ -amylase/g) g for 13 weeks resulted in no treatment related effects.

#### **4.4 Test for Mutagenic Activity of *Bacillus stearothermophilus* $\alpha$ -amylase using *Salmonella typhimurium* strains.**

*Report No. 7899-M-04800* by S. Cinelli and J. Brightwell, Research Toxicology Centre, Roma. Dec 04 2000.

### *Test article*

The test item, *Bacillus stearothermophilus*  $\alpha$ -amylase, was a brown liquid, with an activity of 6540 units/g.

### *Study conduct*

*Bacillus stearothermophilus*  $\alpha$ -amylase was examined for mutagenic activity in five strains of *Salmonella typhimurium* (TA98, TA100, TA102, TA1535 and TA1537). Experiments were performed with or without metabolic action using liver S9 fraction from chemically pre-treated rats. The study design is in accordance with OECD Guidelines<sup>4</sup>.

The study comprised of negative and positive controls with or without S9 metabolising system. Experiments for survival determination and estimation of mutant numbers were carried out in triplicates at each test point.

---

<sup>4</sup> OECD Guideline for the testing of chemicals No. 471 ( Adopted July 1997).

The study comprising was conducted using the direct plate incorporation assay. Five doses of the test substance were applied with 5 mg/plate as the highest dose level followed by successive bi-sections between doses. The test was carried out both in the presence and absence of metabolic activation (in the form of a liver preparation, S-9, and co-factors). The sensitivity of the individual bacterial strains was confirmed by significant increases in the number of revertant colonies induced by diagnostic mutagens (2-Aminoanthracene, 9-Aminoacridine, cumnene hydroperoxide, dimethyl sulfoxide sodium azide and 2-Nitrofluorene).

## 5. Results and conclusion

No dose-related or reproducible increases in mutation frequency were obtained with any of the bacterial strains exposed to  $\alpha$ -amylase either in the presence or absence of metabolic activation. It was concluded that the test material *Bacillus stearothermophilus*  $\alpha$ -amylase did not exhibit any mutagenic activity under the conditions of the test.

### 4.5 Chromosome aberration assay in human lymphocytes cultured *in vitro*

Report No. 7900-M-05100 by S. Cinelli and J. Brightwell, Research Toxicology Centre, Roma. Dec 04 2000.

#### *Test article*

The test item, *Bacillus stearothermophilus*  $\alpha$ -amylase, was a brown liquid, with an activity of 6540 units/g.

#### *Study design*

The potential of *stearothermophilus*  $\alpha$ -amylase to damage the chromosomal structure was tested in an *in vitro* cytogenetics assay, using duplicate human lymphocyte cultures from a healthy male donor. Tests were carried out in the presence and absence of S-9 metabolic activation, over a broad range of doses. The highest dose for chromosome analysis from cultures sampled at 20 hours should be one at which at least 50% mitotic inhibition has occurred or should be the highest dose tested.

#### *Results and conclusion*

Treatment did not produce biologically or statistically significant increases in the frequency of aberrant chromosomes at any concentration tested when compared to control values, either in the presence or absence of S-9 metabolic activation. Positive control, cyclophosphamide, gave the expected increases in the frequency of aberrant metaphases, indicating the efficacy of the metabolic activation mix and the sensitivity of the test procedure.

## 5. Conclusions

This safety assessment of the *Bacillus stearothermophilus*  $\alpha$ -amylase found that:

- the source organism *Bacillus stearothermophilus* has a long history of safe use as a production strain for food-grade enzyme preparations;
- the enzyme preparation complies with JECFA specifications;

- the enzyme preparation produced no evidence of genotoxic potential in *in vitro* assays;
- there was no evidence of toxic effects of *Bacillus stearothermophilus*  $\alpha$ -amylase in the acute and sub-chronic dosing studies in animals.

From the information available, it is concluded that the use of *Bacillus stearothermophilus*  $\alpha$ -amylase as a processing aid in food would pose no public health and safety risk.

## FOOD TECHNOLOGY REPORT

### Introduction

An application was received from Genencor International to amend the *Food Standards Code* to approve the use of the enzyme *alpha*-amylase sourced from *Bacillus stearothermophilus* as a processing aid.

### *Alpha*-amylase

The enzyme class called amylases have had a long history of use in the food industry. They are primarily involved in the hydrolysis of starch. Amylases catalyse the hydrolysis of 1,4-*alpha*-D-glucosidic linkages of polysaccharides such as starch, glycogen, or their degradation products. The specific enzyme, *alpha*-amylase, is termed an endoamylase. It catalyses the endohydrolysis of 1,4-*alpha*-D-glucosidic linkages in polysaccharides, containing three or more 1,4-*alpha*-linked D-glucose units, in a random manner. The term *alpha* ( $\alpha$ ) relates to the optical configuration of the released sugar group, and not to the configuration of the linkage that is hydrolysed (which is random).

In the common usage of *alpha*-amylase the enzyme attacks the random *alpha*-1,4 linkages of amylose and amylopectin of starch, converting them to dextrin so reducing the viscosity and increasing the dextrose equivalent (DE). The *alpha*-amylase enzyme is used to liquefy and dextrinise starch.

### Advantages of use of *alpha*-amylase

Enzymes (in this case *alpha*-amylase) are used to perform specific chemical reactions easier, cheaper and more specifically compared to older chemical reactions relying on inorganic acids and heat to perform reactions. In this case use of *alpha*-amylase is preferred to the use of inorganic acids to perform starch hydrolysis since they have advantages with increased yields and more economic production.

The advantages of using *alpha*-amylase hydrolysis include:

- better specificity of reaction and so reaction products;
- greater control over amylolysis;
- milder reaction conditions (lower temperature and pH conditions) so producing less unwanted side reactions and unwanted off-flavour and odour by-products; and
- the milder reaction conditions are also responsible for more economic production, with lower energy requirements and less neutralisation involved.

The advantages of the *alpha*-amylase derived from *Bacillus stearothermophilus* over other sources (such as *Aspergillus oryzae* and *Bacillus subtilis*) are that it has greater thermostability (enzymic activity maintained at higher temperature) and it produces a different sugar profile.

Alternative sources of the same enzyme will have slightly different properties so which one a manufacturer uses will depend on a number of factors. These properties, being advantages or disadvantages depending on the situation, can be listed as:

- cost;
- temperature sensitivity (temperature dependence on enzymatic activity);
- efficiency of reactions catalysed;
- speed of reaction; and
- reaction products produced (profile).

### **Production of the enzyme**

The *alpha*-amylase is produced using a submerged fed-batch fermentation using *Bacillus stearothermophilus*. The production is standard for many commercially used food enzymes. Good Manufacturing Practice (GMP) is used throughout the production process meeting the requirements and specifications for food enzymes within Food Chemicals Codex (4<sup>th</sup> Edition, 1996) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in the Compendium of Food Additives Specifications, Vol 1, Annex 1 Addendum 9 (2001) (and earlier relevant Addenda).

The Table of Specifications for the enzyme is listed in the Safety Assessment Report (Attachment 3) and so will not be replicated here.

### **Conclusion**

The use of the food enzyme *alpha*-amylase sourced from *Bacillus stearothermophilus* is technologically justified.

### **REFERENCES**

Enzymes in Food Processing, third edition, edited by Tilak Nagodawithana, Academic Press, California (1993).

Food Enzymes, Structure and Mechanism, Dominic Wong, Chapman & Hall, New York (1995).

IUBMB (International Union of Biochemistry and Molecular Biology) Enzyme Nomenclature internet site, <http://www.chem.qmul.ac.uk/iubmb/enzyme/EC3/2/1/1.html>.

Joint FAO/WHO Expert Committee on Food Additives (JECFA) Compendium Addendum 2, FAO Food and Nutrition Paper 52, Addendum 2, page 9 (1993).

Joint FAO/WHO Expert Committee on Food Additives (JECFA), WHO Food Additives Series 28, 37<sup>th</sup> meeting, page 63 (1991).