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

28 October 2008
[18-08]

APPLICATION A1011
CELLULASE FROM *PENICILLIUM FUNICULOSUM* AS A PROCESSING AID (ENZYME)
ASSESSMENT REPORT

Executive Summary

Purpose

Food Standards Australia New Zealand (FSANZ) received an Application from Danisco A/S on 9 July 2008. The Application seeks to amend Standard 1.3.3 – Processing Aids, of the *Australia New Zealand Food Standards Code* (the Code), to approve a cellulase enzyme preparation (EC number 3.2.1.4), produced by fermentation from a non-toxigenic and non-pathogenic strain of *Penicillium funiculosum* as a processing aid.

Processing aids are required to undergo a pre-market safety assessment before approval for use in Australia and New Zealand. Cellulase sourced from *Aspergillus niger*, *Trichoderma reesei* and *Trichoderma viride* are currently listed as permitted processing aids in Standard 1.3.3 – Processing aids in the Table to clause 17 – Permitted enzymes of microbial origin.

It is proposed that this cellulase enzyme preparation will be used in the brewing and distilling industries to allow for more efficient processing and improved product quality whilst also reducing the overall costs of production.

The enzyme preparation meets international specifications for enzymes. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has evaluated and published specifications for the cellulase preparation from *Penicillium funiculosum*. The US Food and Drug Administration (FDA) had affirmed the enzyme as GRAS (Generally Recognized As Safe). It has also been evaluated and accepted for use in Russia and Brazil, and is currently under evaluation in Denmark. This enzyme preparation is currently used in the brewing and distilling industries in Belgium, Germany, Finland, The United Kingdom, Nigeria, Poland, Slovenia, Thailand and Ukraine.

The Application is being assessed under the General Procedure.

Safety Assessment

On the basis of the following considerations, it was concluded that there are no public health and safety concerns associated with the proposed use of cellulase derived from *P. funiculosum*:

- cellulase has very low acute and repeat-dose toxicity in rats, and is not genotoxic *in vitro*;
- there is a history of safe use of the source organism and cellulase in the food industry;
- people are already exposed to both the source organism and cellulase given their natural occurrence in food;
- cellulase derived from a number of other microbial sources is already approved for the same use in Australia and New Zealand; and
- cellulase derived from the same source is an approved processing aid in the USA, Europe and Brazil.

The enzyme is to be used as a processing aid only and any enzyme residues present in the final food product are unlikely to be active and would be metabolised in the gastrointestinal tract in a similar manner to any other ingested protein.

Labelling

The enzyme preparation is produced from a non-genetically modified, classical source organism, *P. funiculosum*. The enzyme cellulase is not considered to be allergenic. The Applicant has not provided an allergen statement in the product specification for identity and purity and there are no potential allergens involved in the manufacturing process. Therefore, there would be no labelling requirement under Standard 1.2.3 for potential allergens.

Assessing the Application

In assessing the Application and the subsequent development of a food regulatory measure, FSANZ has had regard to the following matters as prescribed in section 29 of the *Food Standards Australia New Zealand Act 1991* (FSANZ Act):

- whether costs that would arise from an amendment to the Code to permit the use of the enzyme preparation, cellulase, produced by fermentation from a non-toxigenic strain of *P. funiculosum* would outweigh the direct and indirect benefits to the community, Government or industry;

- there are no other measures that would be more cost-effective than a variation to Standard 1.3.3 that could achieve the same end;

- there are no relevant New Zealand standards; and

- there are no other relevant matters.

Preferred Approach

FSANZ recommends the proposed draft variation to the Table to clause 17 of Standard 1.3.3 – Processing Aids, to permit the use of the enzyme cellulase produced from a non-toxigenic strain of *Penicillium funiculosum* as a processing aid.

Reasons for Preferred Approach

An amendment to the Code approving the use of the cellulase enzyme preparation as a processing aid in Australia and New Zealand is proposed on the basis of the available scientific evidence for the following reasons:

- A detailed safety assessment has concluded that the use of enzyme does not raise any public health and safety concerns.

- The source organism is a classical non-genetically modified strain of *P. funiculosum* and is regarded as non-pathogenic and non-toxigenic.

- Use of the enzyme sourced from *P. funiculosum* is expected to provide technological benefit to food manufacturers.

- The regulation impact assessment has concluded that the benefits of permitting the enzyme as a processing aid outweigh any costs associated with its use.

- There are no other measures that would be more cost-effective than a variation to Standard 1.3.3 that could achieve the same end.

- The proposed draft variation to the Code is consistent with the section 18 objectives of the FSANZ Act.

- There are no relevant New Zealand standards.

Consultation

Public submissions are now invited on this Assessment Report. Comments are specifically requested on the scientific aspects of this Application, in particular, information relevant to the safety assessment of the enzyme cellulase sourced from *P. funiculosum*.

As this Application is being assessed under the general procedure, there will be one round of public comment. Submissions to this Assessment Report will be used to develop the Approval Report for the Application.

Invitation for Submissions

FSANZ invites public comment on this Report and the draft variation to the Code based on regulation impact principles 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 further considering 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, separate it from your submission and provide justification for treating it as confidential commercial material. 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. 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. Alternatively, you may email your submission directly to the Standards Management Officer at submissions@foodstandards.gov.au. There is no need to send a hard copy of your submission if you have submitted it by email or the FSANZ website. FSANZ endeavours to formally acknowledge receipt of submissions within 3 business days.

DEADLINE FOR PUBLIC SUBMISSIONS: 6pm (Canberra time) 9 December 2008

SUBMISSIONS RECEIVED AFTER THIS DEADLINE WILL NOT BE CONSIDERED

Submissions received after this date will only be considered if 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.

Questions relating to making submissions or the application process can be directed to the Standards Management Officer at standards.management@foodstandards.gov.au.

If you are unable to submit your submission electronically, hard copy 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

Food Standards Australia New Zealand
PO Box 10559
The Terrace WELLINGTON 6036
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INTRODUCTION

Food Standards Australia New Zealand (FSANZ) received an Application from Danisco A/S on 9 July 2008. The Application seeks to amend Standard 1.3.3 – Processing Aids, of the *Australia New Zealand Food Standards Code* (the Code), to permit a cellulase enzyme preparation (EC number 3.2.1.4), produced by fermentation from a non-toxigenic and non-pathogenic classical strain of *Penicillium funiculosum* as a processing aid.

This cellulase enzyme preparation is proposed to be used in the brewing and distilling industries for its potential to allow for more efficient processing and improved product quality whilst also reducing the overall costs of production. In brewing, cellulase is used during the mashing stage to reduce the viscosity of the wort and to improve the separation of the wort from the spent grains. In distilling cellulase is used to reduce the viscosity of the mash and to improve the overall efficiency of manufacture.

1. The Issue / Problem

The Applicant proposes the use of cellulase derived from *P. funiculosum* as a processing aid. 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.

A processing aid used in the course of manufacture of a food must be used at the lowest level necessary to achieve a function in the processing of that food, irrespective of any maximum permitted level specified. Processing aids are prohibited from use in food in Australia and New Zealand unless there is a specific permission for them in Standard 1.3.3. Processing aids (which includes enzymes) are required to undergo a pre-market assessment before they are approved for use in food manufacture in Australia and New Zealand.

Currently there are three cellulase enzymes listed in the Table to clause 17 of Standard 1.3.3, with three already-permitted sources of the enzyme (EC 3.2.1.4) (obtained from; *Aspergillus niger*, *Trichoderma reesei* and *Trichoderma viride*).

An assessment (which includes a safety assessment) of the use of cellulase derived from *P. funiculosum* is required before an approval for its use can be given (i.e. listed in Standard 1.3.3).

2. Current Standard

2.1 Historical background

Cellulases and related enzymes have been found to have a great potential in various biotechnology arenas and are used in the food, brewery, distilling and wine industries as well as in animal feed, agriculture, paper and pulp industries, textile and laundry and in other related applications[1].

[1] Bhat, M.K. (2000) Cellulases and related enzymes in biotechnology. *Biotechnology Advances* 18(5):355-383.

2.2 Current Standard

Standard 1.3.3 regulates the use of processing aids in food manufacturing. The Table to clause 17 – Permitted enzymes of microbial origin of Standard 1.3.3 contains a list of permitted enzymes of microbial origin for use as processing aids.

Clause 1 of Standard 1.3.3 defines a processing aid as:

Processing aid *means a substance listed in clauses 3 to 18, where –*

(a) the substance is used in the processing of raw materials, foods or ingredients, to fulfil a technological purpose relating to treatment or processing, but does not perform a technological function in the final food; and

(b) the substance is used in the course of manufacture of a food at the lowest level necessary to achieve a function in the processing of that food, irrespective of any maximum permitted level specified.

Cellulase sourced from *A. niger*, *T. reesei* and *T. viride* are currently listed as permitted processing aids in Standard 1.3.3 in the Table to clause 17 – Permitted enzymes of microbial origin.

Cellulase from the microbial source organism, *P. funiculosum*, is not currently listed in the Table to clause 17 or any other table in Standard 1.3.3.

2.3 International Regulatory Standards

Commercial cellulase enzyme preparation sourced from *P. funiculosum* complies with internationally recognised specifications for the production of enzymes, specifically the specifications of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)[2] and Food Chemicals Codex[3] specifications for enzyme preparations. Detailed specifications for cellulase from *P. funiculosum* have also been revised and published at the 55th JECFA meeting (2000) superseding tentative specifications prepared at the 31st JECFA meeting (1987). No ADI has been allocated to this enzyme preparation.

Cellulase from *P. funiculosum* is affirmed as Generally Recognised As Safe (GRAS) in the United States. Cellulase from *P. funiculosum* is currently sold and used as a processing aid for the brewing, wine and ethanol industries in the United States, Brazil, Belgium, Germany, Finland, the United Kingdom, Nigeria, Poland, Slovenia, Thailand and Ukraine. This enzyme has been evaluated and accepted in Russia and the Ukraine where each new enzyme needs to be approved and listed as permitted.

Within Europe food enzymes are considered processing aids and are excluded from the Food Additive Framework Directive. France, Denmark and the UK have legislation covering all food-use enzymes.

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[2] JECFA (2006) *Compendium of Food Additive Specifications - General specifications and considerations for enzyme preparations used in food processing*. Joint FAO/WHO Expert Committee on Food Additives. FAO Food and Nutrition Paper Monograph 3., 67th session, 63-67. ftp://ftp.fao.org/docrep/fao/009/a0675e/a0675e00.pdf. Accessed on 16 September 2008.

[3] Committee on Food Chemical Codex (2004) *Food Chemical Codex*. 5th Edition. National Academy of Sciences, Food and Nutrition Board, National Academy Press, Washington DC.

In Denmark, where prior approval is needed before enzymes can be used, this enzyme preparation is currently under evaluation. In other EU countries food enzymes should be proven safe before used in food production according to the General EU law.

2.4 Nature of the Enzyme and Source of Organism

The cellulase enzyme preparation sourced from *P. funiculosum* is a mixture of a number of cellulose degrading enzymes including three endoglucanases, two cellobiohydrolases and a β-glucosidase. These enzymes work by catalysing the cellulolysis (or hydrolysis) of cellulose. Substrates for cellulase are the 1,4-β-D-glucosidic linkages in cellulose, lichenin and cereal β-D-glucans, and the 1,4-linkages in β-D-glucans that also contain 1,3-linkages. The mixture also contains "minor enzyme activities" attributed to β-glucanase, which hydrolyses 1,3- or 1,4-linkages of β-D-glucans; xylanase, which hydrolyses 1,4-β-D-xylosidic linkages in xylans; and glucan endo-1,3-β-D-glucosidase, which hydrolyses 1,3-β-D-glucoosidic linkages in 1,3-β-D-glucans. The presence of these minor enzymes is apparently important for the functioning of the cellulase mixture.

The source microorganism is claimed to be a food grade non pathogenic and non toxigenic strain of *P. funiculosum* developed via classical strain improvements only and is not genetically modified.

2.5 Technological purpose of the enzyme

The cellulase enzyme preparation sourced from *P. funiculosum* is used as a processing aid to catalyse the enzymatic hydrolysis of cellulose and thereby convert celluloses into smaller units such as glucose and other soluble sugar units. This cellulase enzyme preparation is proposed to be used in the brewing and distilling industries for its potential to allow for more efficient processing and improved product quality whilst also reducing the overall costs of production. In brewing cellulase is used during the mashing stage to reduce the viscosity of the wort and to improve the separation of the wort from the spent grains. In distilling cellulase is used to reduce the viscosity of the mash and to improve the overall efficiency of manufacture.

2.6 Labelling issues

As a processing aid, the cellulase enzyme preparation would not be required to be labelled on the final food product.

No allergenic materials (as listed in the Table to clause 4 in Standard 1.2.3) are present in the manufacturing process or in the final cellulase enzyme preparation; consequently there would be no labelling requirement necessitated due to any requirements within Standard 1.2.3.

3. Objectives

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

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

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

- the need for standards to be based on risk analysis using the best available scientific evidence;
- the promotion of consistency between domestic and international food standards;
- the desirability of an efficient and internationally competitive food industry;
- the promotion of fair trading in food; and
- any written policy guidelines formulated by the Ministerial Council[4].

4. Questions to be answered

The key questions to be answered are:

- What is the risk to public health and safety from the use of cellulase derived from the microbial source organism, *P. funiculosum*?
- Are there any risk management measures required to protect public health and safety?
- Does the regulatory impact statement (RIS) conclude that the benefits of permitting use of the enzyme outweigh any costs associated with its use?

RISK ASSESSMENT

5. Risk Assessment Summary

5.1 Safety Assessment

The aim of the safety assessment was to evaluate the safety of cellulase, derived from *P. funiculosum* (strain PF8/403-M), for use as a food processing aid. On the basis of the following considerations, it was concluded that there are no public health and safety concerns associated with the proposed use of cellulase derived from this source:

- cellulase has very low acute and repeat-dose toxicity in rats, and is not genotoxic in vitro;
- there is a history of safe use of the source organism and cellulase in the food industry;
- people are already exposed to both the source organism and cellulase given their natural occurrence in food;
- cellulase derived from a number of other microbial sources is already approved for the same use in Australia and New Zealand; and
- cellulase derived from the same source is an approved processing aid in the US, Europe and Brazil.

[4] In May 2008, the Australia and New Zealand Ministerial Council finalised the Policy Guideline on Addition to Food of Substances other than Vitamins and Minerals. This includes policy principles in regard to substances added for technological purposes such as food additives and processing aids. FSANZ has paid regard to each of these principles in assessing this Application.

On the basis that cellulase is a natural part of the diet and that the small concentrations of cellulase (if any) present in the final food would be metabolised as any other dietary protein, it is unnecessary to set an Acceptable Daily Intake (ADI). Therefore, the ADI can be considered to be 'not specified'.

A full safety assessment is provided in **Attachment 2**.

5.2 Dietary Exposure Assessment of the cellulase enzyme preparation

There are no nutritional or dietary implications in approval of this cellulase preparation since there will be no, or very little, residual inactivated enzyme present in the final food and any residues would be metabolised like any other protein. Extensive dietary modelling is not required for the use of the enzyme since it will be used as a processing aid and the majority of the enzyme will be removed from the final product.

5.3 Technological Justification

The use of the cellulase enzyme preparation sourced from *P. funiculosum* as a processing aid is technologically justified to catalyse the enzymatic hydrolysis of cellulose and thereby convert celluloses into glucose and soluble sugars.

The use of the cellulase enzyme would be advantageous in the production of beer to hydrolyse excess β-glucans and ultimately reduce the viscosity of the wort. In the distilling industry the addition of cellulase degrades non-starch polysaccharides such as β-D-glucans and arabinoxylans, resulting in a decrease in mash viscosity, more efficient processing and a potential increase in alcohol yields.

5.4 Production of the enzyme

The cellulase enzyme preparation is produced via a submerged fermentation process utilising appropriate substrate and nutrients under appropriate manufacturing practices. After the fermentation process is complete, the biomass is removed by centrifugation and filtration processes. The remaining fermentation broth, containing the enzyme, is subsequently concentrated.

This concentrated enzyme solution is standardised and stabilised using a compound such as sodium benzoate. A polish filtration step is used as a finalising step for the cellulase preparation. Throughout the manufacturing process, cellulase is monitored and carefully controlled by analytical and quality assurance procedures which ensure that the final product complies with the appropriate specifications and is of the highest quality for use as a processing aid in food manufacturing applications.

5.5 Allergenicity

No allergenic materials (as listed in Table to clause 4 in Standard 1.2.3) have been declared or are found to be present in the manufacturing process or in the final enzyme preparation.

The cellulase enzyme preparation itself is not considered to be allergenic.

RISK MANAGEMENT

6. Issues raised

6.1 Risk Management Strategy

This Assessment Report concludes that the use of cellulase sourced from *P. funiculosum* as a processing aid does not pose a public health and safety risk and its use is technologically justified.

7. 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. The benefits and costs associated with the proposed amendment to the Code have been analysed using regulatory impact principles.

Enzymes (as processing aids in the Code) used in Australia and New Zealand are required to be listed in Standard 1.3.3, and it is not appropriate to consider non -regulatory options.

Two regulatory options have been identified for this Application:

Option 1 Reject the Application

Option 2 Amend Standard 1.3.3 – Processing Aids to permit the use of cellulase sourced from *P. funiculosum* as a processing aid.

8. Impact Analysis (RIS ID: 9852)

In developing food regulatory measures for adoption in Australia and New Zealand, FSANZ is required to consider the impact of all options on all sectors of the community, including consumers, the relevant food industries and governments. The regulatory impact assessment identifies and evaluates, though is not limited to, the costs and benefits arising from the regulation and its health, economic and social impacts. The regulatory impact analysis is designed to assist in the process of identifying the affected parties and the likely or potential impacts the regulatory provisions will have on each affected party. Where medium to significant competitive impacts or compliance costs are likely, FSANZ will seek further advice from the Office of Best Practice Regulation to estimate compliance costs of regulatory options. FSANZ has conducted and OBPR has subsequently approved an assessment of this Application which has concluded that there was no business compliance costs involved and/or minimal impact and consequently a Regulation Impact Statement (RIS) is not required.

8.1 Affected Parties

The affected parties to this Application include the following:

- the enzyme manufacturers and suppliers
- consumers of beer and distilled alcoholic beverages
- food industry, including importers of food, wishing to produce and market food/beverage products manufactured using this enzyme

- the Governments of Australia (Federal, State and Territory) and New Zealand

8.2 Benefit Cost Analysis

8.2.1 Option 1 – Reject the Application

This option is the *status quo*, with no changes to the Code.

Rejecting the Application would disadvantage consumers and relevant food industries where the enzyme could provide a technological function.

8.2.2 Option 2 – Permit the use of cellulase sourced from P. funiculosum as a processing aid.

This option does provide positive benefits to consumers and the food industry. The brewing and distilling industries will benefit from having an alternative source of the cellulase enzyme, which improve process efficiencies and product quality whilst reducing the overall costs of production. The use of the enzyme does not raise any public health and safety concerns.

There should not be any significant compliance costs for government enforcement agencies since they would not need to analyse for the presence of the enzyme. The use of enzymes to treat food during their manufacture does not require labelling so it would not be expected that enforcement agencies would need to analyse for the presence or otherwise of the enzyme in any final food for compliance. There should also be no added costs to consumers.

8.3 Comparison of Options

In assessing Applications, FSANZ considers the impact of various regulatory (and non-regulatory) options on all sectors of the community, including consumers, food industries and governments in Australia.

For this Application, Option 1, the *status quo*, is considered unacceptable because it rejects a technologically justified processing aid as an alternative source of the currently permitted and used processing aid.

Option 2 is favoured since there are potential benefits for the food manufacturing industry, as well as consumers. Such benefits are most likely to include providing manufacturers with an alternative source of the enzyme. No significant adverse costs have been identified with option 2 for government stakeholders. Overall, the benefits outweigh the costs.

COMMUNICATION AND CONSULTATION STRATEGY

9. Communication

FSANZ acknowledges that this Application will be of interest to a broad range of stakeholders and has applied a general communication strategy. This will involve advertising the availability of assessment reports for public comment in the national press and making reports available on the FSANZ website. It is considered that this Application is a routine matter.

The Applicant and individuals and organisations that make submissions on this Application will be notified at each stage of the assessment of the Application.

If approval is recommended, once the FSANZ Board has approved the variation to the standard, that decision will be notified to the Ministerial Council. The Applicant and stakeholders, including the public, will be notified of the gazettal of changes to the Code in the national press and on the FSANZ website.

FSANZ will continue to monitor media response and reaction to this Application. FSANZ also provides an advisory service to the jurisdictions on changes to the Code.

10. Consultation

10.1 Public consultation

FSANZ is seeking comments from the public and other interested stakeholders to help us assess this Application. Once the public comment period has closed, there will be no further round of public comment.

Comments on the following topics would be useful:

- safety considerations
- technological justification
- other scientific aspects
- costs and benefits

10.2 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.

Amending the Code to approve cellulase as a processing aid is unlikely to have a significant effect on trade. The enzyme preparation is consistent with the international specifications for food enzymes of JECFA and the Food Chemicals Codex so there does not appear to be a need to notify the WTO. For these reasons FSANZ has decided not to notify the WTO under either the Technical Barriers to Trade (TBT) or Sanitary and Phytosanitary Measures (SPS) Agreements.

CONCLUSION

11 Conclusion and Preferred Option

This Application has been assessed against the requirements of section 29 of the FSANZ Act. FSANZ recommends the proposed draft variation to Standard 1.3.3.

This Assessment Report concludes that the use of the cellulase enzyme sourced from *P. funiculosum* as a processing aid is technologically justified and does not pose a public health and safety risk.

An amendment to the Code to give approval to the use of cellulase sourced from *P. funiculosum* as a processing aid in Australia and New Zealand is recommended on the basis of the available scientific information. The proposed draft variation is provided in **Attachment 1**.

Preferred Approach

FSANZ recommends the proposed draft variation to the Table to clause 17 of Standard 1.3.3 – Processing Aids, to permit the use of the enzyme cellulase produced from a non-toxigenic strain of *Penicillium funiculosum* as a processing aid.

11.1 Reasons for Preferred Approach

An amendment to the Code approving the use of the cellulase enzyme preparation as a processing aid in Australia and New Zealand is proposed on the basis of the available scientific evidence for the following reasons:

- A detailed safety assessment has concluded that the use of enzyme does not raise any public health and safety concerns.
- The source organism is a classically bred non-genetically modified strain of *P. funiculosum* and is regarded as non-pathogenic and non-toxigenic.
- Use of the enzyme sourced from *P. funiculosum* is expected to provide technological benefit to manufacturers.
- The regulation impact assessment has concluded that the benefits of permitting the enzyme as a processing aid outweigh any costs associated with its use.
- There are no other measures that would be more cost-effective than a variation to Standard 1.3.3 that could achieve the same end.
- The proposed draft variation to the Code is consistent with the section 18 objectives of the FSANZ Act.
- There are no relevant New Zealand standards.

12. Implementation and Review

Following the consultation period for this document, an Approval Report will be completed and the draft variation will be considered for approval by the FSANZ Board. The FSANZ Board's decision will then be notified to the Ministerial Council. Following notification, the proposed draft variation to the Code is expected to come into effect on gazettal, subject to any request from the Ministerial Council for a review of FSANZ's decision.

ATTACHMENTS

1. Draft variation to the *Australia New Zealand Food Standards Code*
2. Safety Assessment Report
3. Food Technology Report

Attachment 1

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

Subsection 87(8) 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 1.3.3*** *of the Australia New Zealand Food Standards Code is varied by inserting the following* Source *in* Column 2 *of the* Table to clause 17 *for the enzyme* Cellulase *in* Column 1 –

Penicillium funiculosum

Attachment 2

Safety Assessment Report

A1011 – Cellulase from *Penicillium funiculosum* as a processing aid (Enzyme)

Summary and Conclusions

The aim of the current assessment was to evaluate the safety of cellulase, derived from *Penicillium funiculosum* strain PF8/403-M, for use as a food processing aid. On the basis of the following considerations, it was concluded that there are no public health and safety concerns associated with the proposed use of cellulase derived from this source:

- cellulase has very low acute and repeat-dose toxicity in rats, and is not genotoxic in vitro;
- there is a history of safe use of the source organism and cellulase in the food industry;
- people are already exposed to both the source organism and cellulase given their natural occurrence in food;
- cellulase derived from a number of other microbial sources is already approved for the same use in Australia and New Zealand; and
- cellulase derived from the same source is an approved processing aid in the US, Europe and Brazil.

On the basis that cellulase is a natural part of the diet and that the small concentrations of cellulase (if any) present in the final food would be metabolised as any other dietary protein, it is unnecessary to set an ADI. Therefore the ADI can be considered to be 'not specified'.

Background

Application A1011 seeks approval for the use of the enzyme cellulase, derived from *P. funiculosum* strain PF8/403-M, and intended for use as a processing aid in brewing and distilling. In both cases, cellulase is added during mashing to degrade polysaccharides and decrease the viscosity of the wort or mash.

Cellulase (EC No. 3.2.1.4) derived from three microorganisms, namely *Aspergillus niger*, *Trichoderma reesei* and *Trichoderma viride*, is approved for use as a processing aid in Australia and New Zealand (Standard 1.3.3 of the Code). In addition, a number of *Penicillium* species are already approved as the source organism for a number of other enzymes used as processing aids (e.g. *P. lilacinum* as a source of dextranase; *P. camembertii* as a source of monoacylglycerol lipase; and *P. roquefortii* as a source of triacylglycerol lipase).

Cellulase derived from *P. funiculosum* is generally recognised as safe (GRAS) in the US and Brazil for use in brewing, wine making and distilling. In addition, it is approved for food use in Russia and is sold for use in brewing in Europe.

Cellulase derived from *P. funiculosum* was evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1987 at its 31st Meeting. No acceptable daily intake (ADI) was established because no safety data were available to the committee (WHO 1987). JECFA specifications for cellulase derived from *P. funiculosum* have been established (FAO 2006a).

The current assessment was undertaken to determine the safety of cellulase derived from *P. funiculosum* (strain PF8/403-M), which is not currently approved in the Code.

Hazard Assessment

Chemistry

Information on the physicochemical properties, product specification and impurity profile for cellulase derived from *P. funiculosum* strain PF8/403-M is provided in the Food Technology Report.

Toxicity of cellulase

The Applicant submitted a number of unpublished toxicity studies on cellulase derived from *P. funiculosum* (designated Cellulase 2000L) including a rat acute oral toxicity study and two *in vitro* genotoxicity studies. A rat subchronic feeding study on a concentrated liquid derived from *P. funiculosum* was also submitted. The strain of *P. funiculosum* from which the test materials were derived was not specified. These studies were quality assured and conducted according to principles of Good Laboratory Practice (GLP). In addition, all studies complied with national and/or international test guidelines. The toxicological database was considered adequate for characterising the hazard posed by cellulase derived from *P. funiculosum* strain PF8/403-M.

Acute toxicity

McRae L (1995) Cellulase 2000L: acute oral toxicity to the rat. Lab: Huntingdon Research Centre Ltd, Cambridgeshire, England. Report No. RNP 461a/950521/AC. Sponsor: Rhone Poulec, ABM Brewing and Enzymes Group, Cheshire, England. Unpublished.

Guidelines/GLP: OECD Test Guideline 401 'Acute oral toxicity' (Adopted 24 February 1987); Statement of compliance with OECD, US EPA, Japanese, UK and EC standards of Good Laboratory Practice (GLP); QA Statement.

Experimental: Groups of five male and five female Hsd/Ola:Sprague-Dawley (CD) rats (sourced from Harlan Olac Ltd, Bicester, Oxon, England; 95-124 g bodyweight; 4-7 weeks old) were given a single oral gavage dose of cellulase [sourced from Rhone Poulec, ABM Brewing and Enzymes Group, Cheshire, England; Batch No. WE 540; activity = 2041 units/g solution; specific activity = 35557 units/g total organic solids (TOS)] at a nominal dose of 2000 mg/kg bw. The test material was given as an undiluted liquid as supplied by the sponsor. Rats were housed 5/cage/sex under standard conditions, with food and water available *ad libitum* from approximately four hours post-dose. Rats were observed twice daily for mortalities and clinical signs. Bodyweights were recorded prior to dosing and at days 8 and 15. Survivors were sacrificed on Day 15 then necropsied.

Findings: There were no deaths. Piloerection was observed in all rats within four minutes of dosing and resolved by day 2. Bodyweight gains in all rats were normal. Necropsy revealed no macroscopic abnormalities. The LD_{50} was > 2000 mg/kg bw (equivalent to 4082 units of enzyme activity).

Subchronic toxicity

Jones LJ, Bartlett AJ & Brooks PN (1998) Penicillium funiculosum concentrated liquid: Ninety day repeated dose oral (gavage) toxicity study in the rat. Lab: Safepharm Laboratories Ltd, Derby UK. SPL Project No. 854/004. Sponsor: Rhodia Ltd, Stockport, Cheshire, UK. Unpublished.

Guidelines/GLP: OECD Test Guideline 408 'Repeated dose 90-day oral toxicity study in rodents'; Statement of compliance with OECD standards of GLP; QA Statement.

Experimental: *P. funiculosum* concentrated liquid (provided by the sponsor; Batch No. TSL35/97; activity = 3823 U/g solution; specific activity = 47137 U/g TOS) was diluted in water and given to groups of 10 Crl:CD®BR rats/sex/dose (sourced from Charles River Ltd, Margate, Kent, UK; 7-8 weeks old; bodyweight range of 158-221 and 142-199 g in males and females, respectively) by oral gavage at nominal doses of 0, 50, 250 or 1000 mg/kg bw/day for 90 days. The dose selection was based on the results of an unpublished 14-day ranging-finding study. The dose volume was 5 mL/kg bw. Rats were housed in groups of up to four/sex under standard conditions, with food and water available *ad libitum*.

Observations for mortalities and clinical signs were made twice daily. Bodyweights were recorded the day prior to the commencement of dosing and thereafter on a weekly basis. Food consumption of each caged group was recorded weekly. Water consumption of each caged group was recorded daily. Ophthalmoscopic examinations were performed prior to treatment and during Week 12. Non-fasted blood was obtained from each rat on Day 90 and the standard range of haematology and clinical chemistry parameters analysed. No urinalysis was performed. All surviving rats were killed at the end of the study and necropsied. The following organs were weighed: adrenals, brain, heart, kidneys, liver, ovaries, spleen and testes. The standard range of organs/tissues were histopathologically examined in the control and high-dose groups. Data were analysed using appropriate statistical tests.

Findings: There were no deaths and no treatment-related clinical signs. Mean absolute bodyweights of treated males was lower (4-8%) than control from Day 14, with a concomitant lower bodyweight gain over the same period. Given the relatively small magnitude of the difference, the absence of a dose-response relationship or a similar effect in females, this observation was not considered treatment-related or toxicologically-significant. Food and water consumption, food conversion efficiency (bodyweight gain/food consumption) haematology and clinical chemistry parameters were unaffected by treatment. There were no treatment-related macroscopic or microscopic abnormalities.

Absolute organ weights were unaffected by treatment. In high-dose females, relative brain weights were significantly higher ($p<0.05$) than the control (0.6809$\pm$0.0546% *versus* 0.6318$\pm$0.0369% of bodyweight, respectively). However, this result was not considered toxicologically-significant as it fell within the historical control range in age and sex-matched rats of the same strain (0.4908-0.8044) as provided in the study report. The no observed adverse effect level (NOAEL) was 1000 mg/kg bw/day, the highest dose tested, based on the absence of any toxicological effects.

Genotoxicity

The results of two *in vitro* genotoxicity assays with cellulase (sourced from Rhone Poulec, ABM Brewing and Enzymes Group, Cheshire, England; Batch No. WE 540; activity = 2041 units/g solution; specific activity = 35557 units/g TOS) are summarised in Table 1. The study of Jones and Gant (1995) was in accordance with OECD Test Guideline 471 (Bacterial Reverse Mutation Test), while the study of Adams et al (1995) was in accordance with OECD Test Guideline 473 (*In Vitro* Mammalian Chromosome Aberration Test). Both assays were conducted in the presence and absence of an exogenous source of metabolic activation (9000 g supernatant containing microsomal enzymes prepared from Aroclor™-induced SD rat liver). In both studies, positive and negative controls gave expected results.

Table 1: *In vitro* genotoxicity of cellulase

End point	Test system	Concentration	Result	Reference
Reverse mutation	*Salmonella typhimurium* strains TA98, TA100, TA1535 & TA1537	0-204.10 units/plate	Negative	Jones & Gant (1995) [GLP; QA]
Chromosomal aberration	Human lymphocytes	0-5.86 U/mL	Negative	Adams et al (1995) [GLP; QA]

GLP = statement of compliance with principle of GLP; QA = quality assured

Discussion

P. funiculosum is a common fungi found in soil and food, and which is not known to be pathogenic, toxic or allergenic to humans. Further, there is a safe history of use of *P. funiculosum* strain PF8/403-M, which has been used to produce food-grade enzyme products since 1991 such as β-glucanase, endo-1,3(4)-beta-glucanase, xylanase and laminarase.

The Applicant stated that *P. funiculosum* strain PF8/403-M is non-toxigenic (i.e. does not produce mycotoxins) and that the conditions used to produce cellulase are not conducive to mycotoxin production, although no evidence was given to substantiate either claim. Certain publications cited by the Applicant reported the co-occurrence of *P. funiculosum* in materials that contained mycotoxins, however, other fungi were also present in the samples and therefore it was unclear whether *P. funiculosum* had actually produced the mycotoxins (see Abdel-Hafez et al 1986; el-Maghraby & Abdel-Sater 1993; Magnoli et al 1998).

An independent search of the scientific literature identified some studies suggesting that mycotoxins can be produced by *P. funiculosum*. Two isolates of *P. funiculosum* derived from corn grains produced ochratoxin A and were moderately toxic in a brine shrimp bioassay (50-75% mortality) (Abdel-Mallek et al 1993). One of three isolates of *P. funiculosum* isolated from mouldy pecan nuts caused reduced growth rates in chicks (Doupnik & Bell 1971). A subsequent study found that two isolates derived from weevil-damaged chestnuts were toxic to chicks, causing tremors and mortality (Wells & Payne 1975). More recent evidence indicated that *P. funiculosum* does not produce mycotoxins (Kozlovsky et al 1999; Frisvad et al 2004; Labuda & Tančinová 2006). The European Food Safety Authority (EFSA 2006) reported that *P. funiculosum*, in general, produces no known mycotoxins except for a single strain that produces secalonic acid. EFSA concluded that *P. funiculosum* is not eligible for a Qualified Presumption of Safety (QPS) because it produces 'many extrolites of unknown structure and biological activity'.

Antimicrobial compounds derived from *P. funiculosum* have been known for some time and include helenine (an antiviral ribonuclear protein) (Shope 1948), funiculosin (an antibiotic) (Ando et al 1969 & 1978) and 4-diazo-3-methoxy-2,5-cyclohexadien-1-one (an antibiotic) (Singh et al 1986). *P. funiculosum* strain No. 8974 isolated from soil was reported to produce a novel non-steroidal aromatase inhibitor, TAN-931 (Ishi et al 1991). In the absence of any information beyond the isolation and basic characterisation of these compounds, it appears that these compounds have found no clinical use in either animal or human medicine.

The weight-of-evidence clearly indicates that *P. funiculosum* strain PF8/403-M is unlikely to be toxigenic. An added assurance of safety is that the impurity limits provided by the Applicant, which are compliant with international standards, indicated that no *P. funiculosum* or mycotoxins are present in the final cellulase product.

The cellulase protein is also widespread in the environment due to its production by bacteria, fungi and plants. It is a reasonable expectation that people are already widely exposed to cellulase as a natural part of their diet. Further, there is a safe history of use of cellulase in the food, animal feed and pharmaceutical industries (TGA 2004). In particular, cellulase derived from a number of microbial sources is already approved in the Code as a food processing aid. Cellulase derived from *P. funiculosum* has GRAS status in the US and Brazil and is sold in Europe for use in brewing. LAMINEX® C2K contains no impurities of toxicological or biological concern such as mycotoxins or antibiotics.

Cellulase derived from *P. funiculosum* (strain unspecified) had very low acute oral toxicity, with the LD_{50} in rats greater than 2000 mg/kg bw (McRae 1995). It was not mutagenic in bacteria (Jones & Gant 1995) and did not cause chromosomal aberrations in human lymphocytes (Adams et al 1995). There was no evidence of toxicity in rats fed a concentrated liquid derived from *P. funiculosum* for 90 days; the NOEL was 1000 mg/kg bw/day, the highest dose tested (Jones et al 1998).

On the basis that cellulase is a natural part of the diet and that the small concentrations of cellulase (if any) present in the final food would be metabolised as any other dietary protein, it is unnecessary to set an ADI. Therefore the ADI can be considered to be 'not specified'.

Conclusion

Based on the available evidence, it is concluded that there are no public health and safety concerns associated with the use of cellulase derived from *P. funiculosum* strain PF8/403-M as a processing aid. The ADI can be considered to be 'not specified'.

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Attachment 3

Food Technology Report

A1011 – Cellulase from *Penicillium funiculosum* as a processing aid (Enzyme)

Summary

Danisco A/S submitted an Application to amend Standard 1.3.3 – Processing Aids of the *Australia New Zealand Food Standards Code* (the Code) to permit a new cellulase enzyme preparation (EC number 3.2.1.4). This enzyme is sourced via a fermentation process from a non-toxigenic and non-pathogenic classical strain of *Penicillium funiculosum.*

This enzyme preparation and its source are requested to be permitted as an approved processing aid in the Table to clause 17 – Permitted enzymes of microbial origin of Standard 1.3.3. The approval of the cellulase preparation from this source would provide an alternative to the currently permitted sources of cellulase already in the Code, including *Aspergillus niger*, *Trichoderma reesei* and *Trichoderma viride*.

This cellulase enzyme preparation is proposed to be used in the brewing and distilling industries. In the brewing industry, the enzyme is claimed to reduce the viscosity of the wort and improve the separation of the wort from the spent grain. In the distilling industry this enzyme is claimed to reduce the mash viscosity and ultimately to improve the overall processing efficiency. The use of this cellulase enzyme preparation would be advantageous and is technologically justified in the brewing and distilling industries.

Introduction

The number of food enzymes and the microbial species from which they are produced has greatly expanded in recent times, partially in response to the constantly evolving and expanding requirements of the international food industry (Pariza and Johnson, 2001). Microbial enzyme preparations have been widely used for a variety of purposes in the production of numerous food products for many years. Their practical application in fermented products dates back many centuries, long before the nature and function of enzymes or even the microorganisms themselves, were known or understood (Bhat, 2000). Cellulases and related enzymes have great biotechnology potential and are used in the food, brewery, distilling and wine industries as well as in animal feed, agriculture, paper and pulp industries, textile and laundry and in other related applications (Beguin and Aubert, 1994; Bhat, 2000; Bayer *et al*., 2007).

Danisco A/S submitted an Application to amend Standard 1.3.3 – Processing Aids to permit a new cellulase enzyme preparation (EC number 3.2.1.4), produced by fermentation from a non-genetically modified, non-toxigenic and non-pathogenic strain of *P. funiculosum* as a processing aid. Various cellulase enzyme preparations (EC 3.2.1.4) are currently listed as permitted processing aids in the Table to clause 17 of the code, these enzyme preparations are obtained from the following sources; *A. niger*, *T. reesei* and *T. viride*.

The Applicant proposes that this cellulase enzyme preparation can be used as a processing aid in the brewing and distilling industries. In the brewing industry the enzyme is claimed to reduce the viscosity of the wort and to improve the separation of the wort from the spent grain. In the distilling industry, use of the enzyme is claimed to reduce the viscosity of the mash and improve the overall processing efficiency.

This commercial cellulase preparation is claimed to comply with internationally recognised specifications for the production of enzymes, specifically the specifications of the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2006) and Food Chemicals Codex (Committee on Food Chemical Codex, 2004) specifications for enzyme preparations. Detailed specifications for cellulase from *P. funiculosum* have also been revised and published at the 55th JECFA meeting (JECFA, 2000).

Characterisation of the enzyme

This cellulase enzyme preparation sourced from *P. funiculosum* is claimed to be a mixture of a number of cellulose degrading enzymes. Its action is a result of the synergistic effect the main and side activities of all the individual enzymes present including endoglucanases, cellobiohydrolases and beta glucosidase. The enzyme is standardised on the basis of its cellulase activity on carboxymethylcellulose (CMC).

Reaction

The main reaction of cellulase is the endohydrolysis of 1, 4-beta-D-glycosidic linkages in polysaccharides such as cellulose, lichenin and cereal beta-glucans, yielding beta-dextrins (JECFA 2000; IUBMB, 2001). The Applicant claims it will also hydrolyse 1,4-linkages in β-D-glucans that also contain 1,3-linkages.

Other minor activities involving the other minor enzymes present include beta glucosidases enzymes working via the endohydrolysis of 1,3- or 1,4-linkages in ß-D-glucans; xylanase catalysing the endohydrolysis of 1,4-ß-D-xylosidic linkages in xylans; and glucan endo-1,3-ß-D-glucosidase catalysing the hydrolysis of 1,3-ß-D-glucoosidic linkages in 1,3-ß-D-glucans.

Identity, purity and specifications of the enzyme

(a) Identity

The common name of the enzyme derived from *P. funiculosum* is 'cellulase'. Other names used include endoglucanase, endo-1,4-betaglucanase, carboxymethylcellulase, endo-1,4-beta-D-glucanase, beta-1,4-glucanase, beta-1,4-endoglucanhydrolase, celludextrinase and avicelase (IUBMB, 2001). This enzyme is produced via fermentation utilising a selected classical strain of *P. funiculosum*.

- Commercial name: LAMINEX® C2K (LAMINEX ® C2000)
- Systematic name: 1,4-(1,3;1,4)-ß-D-glucan 4-glucanohydrolase
- EC number: 3.2.1.4
- CAS number: 9012-54-8

This cellulase enzyme preparation derived from *P. funiculosum* also contains other minor enzyme activities that are essential to the applications of this enzyme preparation. These include ß -glucanase (EC 3.2.1.6), xylanase (EC 3.2.1.8), and glucan endo-1,3-ß-D-glucosidase (EC3.2.1.39).

(b) Purity

Appropriate Good Manufacturing Practices (GMP) controls are used in the manufacture of cellulase preparation from *P. funiculosum* with the following specification for impurities and microbial limits listed in Table 1; these fall well within the JECFA specification (JECFA 2000).

This is to ensure the final treated food product does not contain any impurities of a hazardous or toxic nature.

JECFA has evaluated the cellulase preparation from *P. funiculosum* at the 31st JECFA meeting (1987). The specifications have been published in Food and Nutrition Paper 38 (1988) and have been revised at the 55th JECFA meeting (2000). The revised specifications have been published in JECFA specification FNP 52 Addenda 8 (2000).

This commercial cellulase preparation complies with internationally recognised specifications for the production of enzymes, specifically with the Food Chemicals Codex (5th Edition, 2004) and the FAO Food and Nutrition Paper, Monograph 3 - 'General Specification for Enzyme Preparations used in Food Processing', FAO Food and Nutrition Paper (JECFA, 2006). These specifications referenced are both primary sources of specifications listed in clause 2 of Standard 1.3.4 – Identity and Purity.

Table 1: Specification for Cellulase preparation from *P. funiculosum*

Criteria	Specification for cellulase preparation from *P. funiculosum*
Metals	
Heavy metals as Pb	less than 30 mg/kg
Arsenic	less than 3 mg/kg
Cadmium	less than 0.50 mg/kg
Mercury	less than 0.50 mg/kg
Lead	Not more than 5 ppm
Microbiological	
Total viable count	less than 5 $x10^4$ /ml
Total coliforms	less than 30 /ml
E. coli	absent in 25ml
Salmonella	absent in 25ml
Antibiotic activity	negative by test
Production strain	Absent
Other Assays	
Mycotoxins	Negative
Minimum activity	3150 CMC U/g*
Physical Properties	
pH	3.7 – 4.2
Appearance	non viscous, clear, light brown liquid
Temperature optimum	approximately 55ºC
Thermal stability	not stable above 60 ºC
pH optimum	approximately pH 5
pH stability	4 – 7

*****The activity of the cellulase enzyme preparation is expressed in carboxymethylcellulose activity units (CMC U). One CMC unit of activity liberates 1 µmol of reducing sugars (expressed as glucose equivalents) in one minute under specific assay conditions (50°C and pH 4.8).

Enzyme production

The cellulase enzyme preparation is produced via a submerged fermentation process utilising appropriate substrates and nutrients under good manufacturing practices.

After the fermentation process is complete, the biomass is removed by centrifugation and filtration processes. The remaining fermentation broth, containing the enzyme, is subsequently concentrated. This concentrated enzyme solution is standardised and stabilised using appropriate additives such as sodium benzoate. A polish filtration step is used as a purification step in cellulase manufacture.

Throughout the manufacturing process, cellulase is monitored and carefully controlled by analytical and quality assurance procedures which ensure that the final product complies with the appropriate specifications as listed above and is suitable for use as a processing aid in food processing applications.

Source organism

The source microorganism is claimed to be a food grade non pathogenic and non toxigenic strain of *Penicillium funiculosum* developed via classical strain improvements only and is not genetically modified.

Technological function of the enzyme / Applications

Active research on the uses of cellulase enzymes was being conducted as early as the 1950's on their potential to convert celluloses into glucose and soluble sugars and these enzyme preparations have been actively researched since this time (Schwardt, 1990; Bhat, 2000). Cellulases have been shown to have enormous potential for use in food biotechnology arenas. Uses for these cellulose degrading enzymes include the extraction and clarification of fruit and vegetable juices, the production of fruit nectars and purees, in the improvement of the sensory properties of fruits and vegetables extracts, extraction of olive oil and to improve the quality of bakery products. Cellulases have also been extensively utilised in the winery, brewing and distilling industries (Bhat, 2000).

The biological degradation of cellulose has been thoroughly reviewed by a number of authors and the literature generally indicates that the enzymatic hydrolysis of cellulose by cellulase enzymes are highly specific reactions (Gams, 1976; Schwardt, 1990; Beguin and Aubert, 1994; Bhat, 2000). Cellulase enzyme preparations are primarily utilised to catalyse the enzymatic hydrolysis of cellulose. Cellulase enzyme preparations are often a complex mixture of several enzymes all intimately involved in the hydrolysis of cellulose (Mussatto *et al*., 2006). The performance of this particular enzyme preparation is a result of the synergistic effects of cellulase, endoglucanases, cellobiohydrolases and beta glucosidase enzymes present.

Another recent study by Karboune *et al*., (2008) investigated the characterisation of selected celluloytic activities of a multi-enzymatic preparation produced from *P. funiculosum*, and found an interesting feature was the synergistic action of the enzymes working together for the hydrolysis of native cellulose into individual glucose units. In this study *P. funiculosum* was noted as being widely recognised as an important source of highly active multi-cellulolytic preparation systems capable of degrading cellulose in a number of practical situations.

In a recent study by Mussatto *et al*.,(2008) the effects of the presence of hemicellulose and lignin concentrations on the enzymatic hydrolysis of cellulose obtained from brewer's spent grain was further characterised. Uses of brewer's spent grain extend to various bio-processing arenas including energy production, biotechnology, animal feed and as human nutritional adjuncts. The authors concluded that the less hemicellulose and lignin content in the brewers' grain the greater the efficiency of the cellulase enzyme (Mussatto *et al*., 2008).

(a) Beer

Beer production has an ancient history and has been conducted for many centuries. Essentially the production of beer involves brewing and fermenting the starches derived from cereals grains. Grain undergoes malting followed by the addition of water and the preparation and fermentation of the wort. Barley is the most common cereal used for the production of beer although wheat, corn, and rice are also widely used.

Beer is often flavoured with hops, which is utilised to impart a bitter taste to the final product. Other adjuncts such as sugars may also be added. The main processes involved in beer production include milling to reduce the size of the dry malt in order to increase availability of carbohydrates, mashing where water is added to the malt, lautering or filtration where spent grains are removed form the wort, boiling the wort with flavouring hops, fermentation of the wort liquor, maturation, conditioning, filtration and packaging of the final product.

Enzyme technology and interaction can play a key role in the production of beer. The addition of cellulases and other polysaccaridases are known to both improve the quality of the final product as well as increase overall productivity of the manufacturing process.

Endogenous enzyme production generally occurs naturally during the malting stage of beer production; which involves the barley grain (and/or other cereal grains) being held under conditions which encourage the grain to germinate. The biosynthesis and activation of endogenous enzymes (e.g. alpha and beta amylases, carboxypeptidase and beta glucanase) serve to hydrolyse the seed's carbohydrate reserves. In an ideal world this endogenous mixture of enzymes should work synergistically under optimal conditions to produce consistent high quality malted wort liquid. In reality however, due to seasonal variations in the grain, poor harvests and/or using a combination of different cultivars together, the wort, may potentially contain un-malted grains along with an excess amount of high molecular weight polysaccharides such as beta glucans (Bhat, 2000). This excess of beta glucans has the potential to increase mash viscosity and cause haze formations, poor dewatering, low yields, slow run off time, and lead to poor filterability of the wort and the development of an unwanted haze in the final beer. The addition of a commercial cellulase enzyme preparation (specifically containing beta glucanases) at the mashing stage can serve to hydrolyse excess beta glucans and ultimately reduce the viscosity of the wort (Bhat, 2000).

The Applicant states that this cellulase enzyme preparation will be used during the mashing stage to reduce the viscosity of the wort and subsequently improve the separation of the wort from the spent grain. The cellulase enzyme preparation degrades beta glucans and arabinoxylans which are extracted during the mashing process and thereby reduces the viscosity of the wort and improves the separation of the wort from the spent grains. The level of enzyme use proposed is 10-100 mg of active enzyme per kg of cereal.

(b) Distilling

The distilling process involves four main steps including milling to reduce the size of the malted grain, mashing where water is added to the malt, fermentation and the final distillation of the spirit. The most common grains used in distilling beverages include wheat and barley.

The Applicant states that this cellulase enzyme preparation will be added during the mashing step to degrade non-starch polysaccharides such as beta glucans and arabinoxylans, resulting in a decrease in mash viscosity, more efficient processing, including a reduction in the amount of fuel required due to an improvement in heat transfer, and a potential increase in alcohol yields. The level of enzyme use proposed by the Applicant is between 10-100 mg of active enzyme per kg of grist. The Applicant also states the enzyme will only be used in the production of white spirits.

Allergenicity

Given the manufacturing process and ingredients described above and within the Application, no allergenic materials (as listed in the Table to clause 4 of Standard 1.2.3) are present in the manufacture or final enzyme preparation.

The cellulase enzyme itself is not considered to be allergenic.

Conclusion

The use of the cellulase enzyme preparation, sourced from *P. funiculosum*, as a processing aid is technologically justified to catalyse the enzymatic hydrolysis of cellulose and other polysaccharides. The use of the cellulase enzyme preparation would be advantageous and technologically justified in the brewing and distilling industries.

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