Protein-glutamine glutaminase from *Bacillus licheniformis*

An application to amend the Australia New Zealand Food Standards Code with a protein-glutamine glutaminase preparation produced by a genetically modified strain of *Bacillus licheniformis*

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Executive summary

The present application seeks to amend Schedule 18—Processing aids of the Australia New Zealand Food Standards Code (the Code) to approve a protein-glutamine glutaminase enzyme preparation produced by Novozymes.

Proposed change to Australia New Zealand Food Standards Code – Schedule 18—Processing aids

Schedule 18—Processing aids is proposed to be amended to include a genetically modified strain of *Bacillus licheniformis* expressing a protein-glutamine glutaminase from *Chryseobacterium viscerum* as permitted source for protein-glutamine glutaminase.

The application is applied for assessment by the general procedure.

Description of enzyme preparation

The enzyme is a protein-L-glutamine amidohydrolase (EC 3.5.1.44), commonly known as protein-glutamine glutaminase.

Protein-glutamine glutaminase catalyses deamidation of gamma-amide of peptide-bound glutamine to form peptide-bound glutamate with subsequent release of ammonia.

The enzyme is produced by submerged fermentation of a *Bacillus licheniformis* microorganism expressing a protein-glutamine glutaminase from *Chryseobacterium viscerum*.

The protein-glutamine glutaminase enzyme preparation is available as a liquid preparation complying with the JECFA recommended purity specifications for food-grade enzymes.

The producing microorganism, *Bacillus licheniformis*, is absent from the commercial enzyme product.

Use of the enzyme

The protein-glutamine glutaminase enzyme preparation is used in the following food manufacturing processes:

- Production of heat-treated milk and milk-based barista products
- Production of cheese
- Production of fermented milk products
- Production of modified milk protein
- Production of plant-based analogues of milk and milk products
- Production of protein hydrolysates from plants and fungi
- Processing of yeast and yeast products
- Production of protein hydrolysates from meat and fish proteins
- Production of plant-based meat analogues

Benefits

The benefits of the use of the food enzyme in these food manufacturing processes are summarised below.

Production of heat-treated milk and milk-based barista products

- reduce fouling in heat treatment equipment
- reduce layer separation of UHT milk
- improve foaming capacity and foam texture in low fat milk used in barista products
- reduce curding and improve mouth feel of cooled ready-to-drink milk-based coffee products

Production of cheese

- increased yield in cheese production
- improved texture
- reduced syneresis upon storage

Production of fermented milk products

- increased viscosity and firmness of fermented milk products particularly in low fat products
- reduced syneresis (whey separation)

Production of modified milk protein

- reduced fouling in production equipment
- improved solubility of milk proteins
- higher heat tolerance of milk proteins

Production of plant-based analogues of milk and milk products

- reduced fouling in production equipment
- increased protein solubility
- reduced risk of curding for plant-based beverage added to coffee/tea
- less viscosity and better texture

Production of protein hydrolysates from plants and fungi

- increase protein solubility, emulsification capability and gelling ability
- increase the degree of hydrolysis

Processing of yeast and yeast products

- improved process efficiency
- improved solubility and functionality of yeast protein

Production of protein hydrolysates from meat and fish proteins

- source of protein with high solubility, digestibility and/or increased nutritional value
- improved taste, texture and water binding in the final food

- in gelatine extraction, reduced need for acid/base in process, higher yield, improved protein solubility and functionality

Production of plant-based meat analogues

- improved meat-like structure (increased firmness and chewiness)
- improved cutting strength
- increased protein solubility

Safety evaluation

The safety of the production organism and the enzyme product have been thoroughly assessed:

- The production organism has a long history of safe use as production strain for food-grade enzyme preparations and is known not to produce any toxic metabolites.
- The genetic modifications in the production organism are well-characterised and safe, and the recombinant DNA is stably integrated into the production organism and unlikely to pose a safety concern.
- The enzyme preparation complies with international specifications ensuring absence of contamination by toxic substances or noxious microorganisms.
- Sequence homology assessment to known allergens and toxins shows that oral intake of the protein-glutamine glutaminase does not pose food allergenic or toxic concern.

Furthermore, the safety of the protein-glutamine glutaminase preparation was confirmed by external expert groups, as follows:

- Denmark: The enzyme preparation was safety assessed resulting in the authorisation of the enzyme product by the Danish Veterinary and Food Administration.

Conclusion

Based on the Novozymes safety evaluation, confirmed by the above-mentioned bodies, we respectfully request the inclusion of the protein-glutamine glutaminase in Schedule 18—Processing aids.

Introduction

The present application describes a protein-glutamine glutaminase enzyme preparation produced by submerged fermentation of a *Bacillus licheniformis* microorganism producing a protein-glutamine glutaminase from *Chryseobacterium viscerum*.

The enzyme is a protein-glutamine glutaminase (EC 3.5.1.44), commonly known as protein-glutamine glutaminase. In the presence of water, the enzyme catalyses deamidation of gamma-amide of peptide-bound L-glutamine to form peptide-bound L-glutamate with subsequent release of ammonia. The protein-glutamine glutaminase enzyme preparation is intended to be used as processing aid in various food manufacturing processes.

The following sections describe in detail the construction of the genetically modified *Bacillus licheniformis* used as the production organism, the production process, the product specification, the application of the enzyme preparation and finally the safety evaluation of the product.

The documentation has been elaborated according to the Application Handbook from Food Standards Australia New Zealand as of 1 July 2019, applied as relevant for an enzyme application, *i.e.,* outlining the following section:

- Section 3.1.1 – General requirements
- Section 3.3.2 – Processing aids, subsections A, C, D, E, F

NB! In Appendix 2.1, the protein-glutamine glutaminase enzyme preparation is described by its commercial name, Vertera Velvet.

Chapter 3.1, General requirements for applications

A Executive summary

An Executive Summary is provided as a separate copy together with this application.

B Applicant details

(a) **Applicant's name/**

[redacted]

(b) **Company/organisation name**

Chr. Hansen Pty Ltd

(c) **Address (street and postal)**

49 Barry Street, Bayswater,
Victoria, 3153, Australia

(d) **Telephone number**

[redacted]

(e) **Email address**

[redacted]

(f) **Nature of applicant's business**

Biotechnology

(g) **Details of other individuals, companies or organisations associated with the application**

Submission prepared by:

To whom correspondence should be directed:

[redacted]

C Purpose of the application

This application is submitted to provide for amendment of the Australia New Zealand Food Standards Code, Schedule 18—Processing aids to include a genetically modified strain of *Bacillus licheniformis* as permitted source for a protein-glutamine glutaminase.

D Justification for the application

The need for the proposed change

Schedule 18—Processing aids contains a list of permitted enzymes of microbial origin, including another protein-glutamine glutaminase (EC 3.5.1.44) from *Chryseobacterium proteolyticum*. However, Schedule 18—Processing aids does not contain a protein-glutamine glutaminase (EC 3.5.1.44) from *Bacillus licheniformis* containing the gene for protein-glutamine glutaminase from *Chryseobacterium viscerum*.

Bacillus licheniformis is an approved host and production strain for a number of enzymes in Schedule 18—Processing aids, *e.g.* a wide range of enzymes that can be used as processing aids such as alpha-amylase, beta-galactosidase, chymotrypsin, xylanase, maltogenic alpha-amylase, pullulanase, serine proteinase.

The advantages of the proposed change over the status quo

The protein-glutamine glutaminase preparation is used as a processing aid in various food manufacturing processes. Protein-glutamine glutaminases catalyses deamidation of gamma-amide of peptide-bound glutamine.

The benefits of the use of the food enzyme in these food manufacturing processes are summarised below.

Production of heat-treated milk and milk-based barista products

- reduce fouling in heat treatment equipment
- reduce layer separation of UHT milk
- improve foaming capacity and foam texture in low fat milk used in barista products
- reduce curding and improve mouth feel of cooled ready-to-drink milk-based coffee products

Production of cheese

- increased yield in cheese production
- improved texture
- reduced syneresis upon storage

Production of fermented milk products

- increased viscosity and firmness of fermented milk products particularly in low fat products
- reduced syneresis (whey separation)

Production of modified milk protein

- reduced fouling in production equipment
- improved solubility of milk proteins
- higher heat tolerance of milk proteins

Production of plant-based analogues of milk and milk products

- reduced fouling in production equipment
- increased protein solubility
- reduced risk of curding for plant-based beverage added to coffee/tea
- less viscosity and better texture

Production of protein hydrolysates from plants and fungi

- increase protein solubility, emulsification capability and gelling ability
- increase the degree of hydrolysis

Processing of yeast and yeast products

- improved process efficiency
- improved solubility and functionality of yeast protein

Production of protein hydrolysates from meat and fish proteins

- source of protein with high solubility, digestibility and/or increased nutritional value
- improved taste, texture and water binding in the final food
- in gelatine extraction, reduced need for acid/base in process, higher yield, improved protein solubility and functionality

Production of plant-based meat analogues

- improved meat-like structure (increased firmness and chewiness)
- improved cutting strength
- increased protein solubility

The benefits, which are described above, are not exclusively obtainable by means of enzyme treatment but can be achieved through *e.g.* modifying maybe more expensive or less environmentally friendly production processes or recipe changes.

D.1 Regulatory impact information

D.1.1 Costs and benefits of the application

The application is not likely to place costs or regulatory restrictions on industry or consumers. Inclusion of the protein-glutamine glutaminase enzyme in Schedule 18—Processing aids will provide the food and beverage industry with the opportunity to improve processing of several food products under environmentally friendly and cost-efficient production conditions. For the government, the burden is limited to necessary activities for a variation of Schedule 18—Processing aids.

D.1.2 Impact on international trade

The application is not likely to cause impact on international trade.

E Information to support the application

E.1 Data requirements

No public health and safety issues related to the proposed change are foreseen. As outlined in sections 3.3.2 C, D, E, F, the protein-glutamine glutaminase is produced by submerged fermentation of a genetically modified *Bacillus licheniformis* strain.

The safety of the production organism and the enzyme product has been thoroughly assessed:

- The production organism has a long history of safe use as production strain for food-grade enzyme preparations and is known not to produce any toxic metabolites.
- The genetic modifications in the production organism are well-characterised and safe, and the recombinant DNA is stably integrated into the production organism and unlikely to pose a safety concern.
- The enzyme preparation complies with international specifications ensuring absence of contamination by toxic substances or noxious microorganisms.
- Sequence homology assessment to known allergens and toxins shows that oral intake of the protein-glutamine glutaminase does not pose food allergenic or toxic concern.

F Assessment procedure

Because the application is for a new source organism for an existing enzyme in the Code, it is considered appropriate that the assessment procedure is characterised as “General Procedure, Level 1”.

G Confidential commercial information (CCI)

Detailed information on the raw materials used in production of the enzyme preparation and construction and characteristics of the genetically modified production strain are provided in **Appendices 3**, **4** and **6**, respectively. Summaries of the information are given in section A.4 and 3.3.2 E. The formal request for treatment of selected parts of **Appendices 3, 4** and **6** as confidential commercial information (CCI) is included as **Appendix 1.1**.

H Other confidential information

Apart from the selected parts of **Appendices 3, 4** and **6** identified as confidential commercial information (CCI), no other information is requested to be treated as confidential.

I Exclusive capturable commercial benefit (ECCB)

This application is not expected to confer an Exclusive Capturable Commercial Benefit.

J International and other national standards

J.1 International Standards

Use of enzymes as processing aids for food production is not restricted by any Codex Alimentarius Commission (Codex) Standards.

J.2 Other national standards or regulations

With few exceptions on national, commodity standards, use of enzymes as processing aids for food production is in general not restricted by standards or regulations in other countries.

K Statutory declaration

The Statutory Declaration is provided as a separate document together with this submission.

L Checklist

This application concerns an enzyme product intended to be used as a processing aid. Therefore, the relevant documentation according to the Application Handbook from Food Standards Australia New Zealand as of 1 July 2019, are the following sections:

- Section 3.1.1 – General requirements
- Section 3.3.2 – Processing aids, subsections A, C, D, E, F

Accordingly, the checklist for General requirements as well as the Processing aids part of the checklist for applications for substances added to food was used and is included as **Appendix 1.2** and **1.3**.

Chapter 3.3, Guidelines for applications for substances added to food

3.3.2 Processing Aids

The protein-glutamine glutaminase enzyme preparation described in this application is representative of the commercial food enzyme product for which approval is sought.

A Technical information on the processing aid

A.1 Information on the type of processing aid

The protein-glutamine glutaminase enzyme preparation belongs to the category of processing aids described in Schedule 18—Processing aids.

The protein-glutamine glutaminase enzyme preparation is to be used in the food industry as a processing aid during the processing of raw materials containing peptide-bound L-glutamine. In the presence of water, a protein-glutamine glutaminase catalyses deamidation of gamma-amide of peptide-bound L-glutamine to form peptide-bound L-glutamate with subsequent release of ammonia. The properties of use of the food enzyme are technologically beneficial in various industrial food manufacturing processes, e.g. improving the processing of protein, increase protein solubility, emulsifying and foaming properties, and improving their flavour-enhancing properties (Liu et al., 2022).

The protein-glutamine glutaminase enzyme preparation is used in the following food manufacturing processes:

- Production of heat-treated milk and milk-based barista products
- Production of cheese
- Production of fermented milk products
- Production of modified milk protein
- Production of plant-based analogues of milk and milk products
- Production of protein hydrolysates from plants and fungi
- Processing of yeast and yeast products
- Production of protein hydrolysates from meat and fish proteins
- Production of plant-based meat analogues

The highest dosage of the protein-glutamine glutaminase during a food manufacturing process is in production of protein hydrolysates from plants and fungi/ production of plant-based meat analogues, where dosages up to 165 mg TOS per raw material (e.g., plant and fungal proteins) are used.

A.2 Information on the identity of the processing aid

A.2.1 Enzyme

Generic name	Protein-glutamine glutaminase
IUBMC nomenclature	Protein-L-glutamine amidohydrolase
IUBMC No.	EC 3.5.1.44
Cas No.	62213-11-0

A.2.2 Enzyme preparation

The enzyme concentrate is formulated into a final enzyme preparation. The enzyme concentrate may be intended for a single enzyme preparation or a blend with other food enzymes and formulated as a liquid product depending on the characteristics of the intended food process in which it will be used.

The typical composition of the enzyme concentrate is:

Enzyme solids (TOS)[1]	approx. 3 %
Sodium chloride	approx. 10 %
Sucrose	approx. 40 %
Water	approx. 47 %

The enzyme concentrate is standardised in protein-glutamine glutaminase units to an activity of 25000 PGLU-A/g. The Novozymes method used to determine the PGLU-A/g activity is enclosed in **Appendix 3.2**.

A.2.3 Host organism

The production strain was developed from the *Bacillus licheniformis* Si3 cell lineage, which was derived from the natural isolate *Bacillus licheniformis* Ca63. The Si3 cell lineage has a long history of safe use at Novozymes for production of food enzymes and has given rise to a number of food enzyme production strains, which are used for production of previously evaluated and regulatory approved food enzymes. The taxonomic classification of the recipient strain is:

[1] TOS = Total Organic Solids, defined as: 100 % - water - ash - diluents

Phylum	Firmicutes
Class	Bacilli
Order	Bacillales
Family	Bacillaceae
Genus	*Bacillus*
Species	*Bacillus licheniformis*

For a more detailed description of the host organism and the genetic modifications, please see section 3.3.2 E.

A.2.4 Donor organism

The donor for the protein-glutamine glutaminase gene is as follows.

Phylum	Bacteroidota
Class	Flavobacteriia
Order	Flavobacteriales
Family	Weeksellaceae
Genus	Chryseobacterium
Species	*Chryseobacterium viscerum*

For a more detailed description of the donor and the donor gene, please see section 3.3.2 E and **Appendix 6**.

A.3 Information on the chemical and physical properties of the processing aid

The enzyme is a protein-glutamine glutaminase (EC 3.5.1.44), commonly known as protein-glutamine glutaminase. In the presence of water, a protein-glutamine glutaminase catalyses deamidation of gamma-amide of peptide-bound L-glutamine to form peptide-bound L-glutamate with subsequent release of ammonia.

The enzyme preparation is available as liquid product. The appearance of the enzyme product is given in **Appendix 2.1**. The micro-organisms used for fermentation will consume the majority of the raw materials. Subsequent down-stream processes, such as washing and filtration steps will remove remaining amounts of raw material. Thus, the final food enzyme product will not contain significant residual amounts of the nutrients used for fermentation. This is confirmed by regular spot-testing, where analytical measurements show no detectable amounts of the allergens listed in **Appendix 2.1**.

The protein-glutamine glutaminase is active at temperatures up to 65 °C (with an optimum of 25-30 °C at pH 7) and within a pH range of 3 to 8 (an optimum of pH 4.0 at 40 °C). The temperature stability data shows that the protein-glutamine glutaminase is completely inactivated after 10 min pre-incubation at 75°C and above. The food processes, in which the enzyme is applied, include processing steps where it is denatured at high temperatures.

The food enzyme object of the present application is not added to final foodstuffs but used as a processing aid during food manufacturing. The enzyme exerts no function in the final food. In the final food the enzyme protein is either denatured by high temperature, which means that it does not have any action or any function, and is thus, like any other protein, inert; and/or removed in certain processing steps.

No reaction products, which could not be considered normal constituents of the diet, are formed during the production or storage of the enzyme-treated food.

A.4 Manufacturing process

The manufacturing process comprises a fermentation process, a purification process, a formulation process and finally a quality control of the finished product, as outlined by Aunstrup (1979). This section describes the processes used in manufacturing of the protein-glutamine glutaminase enzyme product.

The enzyme preparation is manufactured in accordance with current Good Manufacturing Practices (**Appendix 4.1**). The quality management system used in the manufacturing process complies with ISO 9001:2015 (**Appendix 4.2**).

The raw materials are of food-grade quality and have been subjected to appropriate analysis to ensure their conformity with the specifications.

A.4.1 Fermentation

The protein-glutamine glutaminase is produced by submerged fed-batch pure culture fermentation of the genetically modified strain of *Bacillus licheniformis*, described in section 3.3.2 E.

A.4.1.1 Raw materials for fermentation

The production strain is grown in a medium consisting of compounds providing an adequate supply of carbon and nitrogen as well as minerals and vitamins necessary for growth. Furthermore, acids and bases for the adjustment of the pH and processing aids (*e.g.* antifoaming agents) are used during fermentation. The choice of raw materials used in the fermentation process (the feed, the seed fermenter, the main fermenter and dosing) is given in the confidential parts of **Appendix 4.3**.

A.4.1.2 Hygienic precautions

All equipment is designed and constructed to prevent contamination by foreign microorganisms.

All valves and connections not in use for the fermentation are sealed by steam at more than 120 °C.

After sterilization a positive pressure of more than 0.2 atmosphere is maintained in the fermentation tank.

The air used for aeration is sterilised by passing through a sterile filter. The inside of each fermentation tank is cleaned between fermentations by means of a high-pressure water jet and inspected after the cleaning procedures have been completed.

A.4.1.3 Preparation of the inoculum

The inoculum flask containing the prepared medium is autoclaved and checked. Only approved flasks are used for inoculation.

The stock culture suspension is injected aseptically into the inoculum flask. The identification number of the used stock culture vial is registered on the inoculum flask and in the batch documentation.

When sufficient amount of biomass is obtained and when the microbiological analyses are approved, the inoculum flask can be used for inoculating the seed fermenter.

A.4.1.4 The seed fermentation

The raw materials for the fermentation medium are mixed with water in a mixing tank of stainless steel. The mixture is transferred to the seed fermenter and heat sterilised (*e.g.* 120 °C/60 min).

The seed fermentation tank is inoculated by transferring aseptically a suspension of cells from the inoculum flask.

The seed fermentation is run aerobically (sterile airflow), under agitation. The overpressure is kept above 0.2 atmosphere at all times, to prevent contamination.

Once a sufficient amount of biomass has developed, microbiological analyses are performed to ensure absence of contamination. The seed fermentation can then be transferred to the main fermentation tank.

A.4.1.5 The main fermentation

The raw materials for the medium are mixed with water in a mixing tank. The medium is transferred to the main fermenter and heat sterilised (*e.g.* 120 °C/60 min). If necessary, the pH is adjusted after sterilization, with sterile pH adjustment solutions.

The fermentation in the main tank is run as normal submerged fed-batch fermentation.

The main fermentation is run aerobically (sterile airflow), under vigorous agitation. The overpressure is kept above 0.2 atmosphere at all times, to prevent contamination. The fermentation is run at a well-defined temperature.

During the fermentation, sterile fermentation medium is fed aseptically. Feeding is started when pH increases above its set point. The feed rate is controlled by pH fluctuations and adjusted to avoid accumulation of carbohydrates.

At regular intervals the fermentation is controlled for:

- Dissolved oxygen
- pH
- Refractive index
- Microbial contamination
- Enzyme activity

At the end of the main fermentation the culture is inspected by microscopy and samples are taken for microbial analysis. If no observations of unexpected microorganisms are found, the fermentation can then be used for harvest and downstream processing.

A.4.2 Recovery

The recovery process is a multi-step operation designed to separate the desired enzyme from the microbial biomass (production strain) and to partially purify, concentrate, and stabilise the enzyme. The process steps involve a series of unit operations:

- Pre-treatment
- Primary separation
- Filtration
- Concentration
- Polish and germ filtration

A.4.2.1 Raw materials for recovery

The choice of raw materials used during recovery is given in the confidential parts of **Appendix 4.3**.

A.4.2.2 Pre-treatment

To facilitate the separation, flocculants are used in a pH-controlled process.

A.4.2.3 Primary separation

The cell mass and other solids are separated from the broth by well-established techniques such as pre-coat vacuum drum filtration or centrifugation.

The primary separation is performed at well-defined pH and temperature range.

A.4.2.4 Filtration

For removal of residual cells of the production strain and as a general precaution against microbial degradation, filtration on dedicated germ filtration media is applied. Pre-filtration is included when needed.

The filtrations are performed at well-defined pH and temperature intervals, and result in an enzyme concentrate solution free of the production strain and insoluble substrate components from the fermentation.

A.4.2.5 Concentration

Ultrafiltration and/or evaporation are applied for concentration and further purification. The ultrafiltration is applied to fractionate high molecular weight components (enzymes) from low molecular weight components and is used to increase the activity/dry matter ratio.

The pH and temperature are controlled during the concentration step, which is performed until the desired activity and activity/dry matter ratio has been obtained.

Further concentration may be done by evaporation.

A.4.2.6 Polish and germ filtration

As a general precaution against microbial contamination, filtration on dedicated germ filters is applied at various stages during the recovery process. Pre-filtration (polish filtration) is included if needed to remove insoluble substances and facilitate the germ filtration. To facilitate the polish filtration, a filter aid may be added.

The final polish and germ filtration at the end of the recovery process results in a concentrated enzyme solution free of the production strain and insoluble substances.

The enzyme solution before formulation is defined as the “food enzyme”.

A.4.3 Formulation

Subsequently, the food enzyme is formulated into a final enzyme preparation. The food enzyme may be intended for a single enzyme preparation or a blend with other food enzymes and formulated as a liquid product or a granulate depending on the characteristics of the intended food process in which it will be used.

A.4.4 Quality control

The manufacturing process of the food enzyme includes a fermentation process, a recovery process, a formulation process and finally a quality control of the finished product, as outlined by (Aunstrup, 1979).

After final formulation the food enzyme preparation is controlled and analysed in accordance with the general specifications for enzyme preparations used in food processing as established by JECFA (FAO/WHO, 2006).

A.5 Specification for identity and purity

The protein-glutamine glutaminase enzyme product complies with the purity criteria recommended for Enzyme Preparations in Food, Food Chemicals Codex, 11th edition, 2018.

In addition to this, the protein-glutamine glutaminase enzyme product also conforms to the General Specifications for Enzyme Preparations Used in Food Processing as proposed by the Joint FAO/WHO Expert Committee on Food Additives in Compendium of Food Additive Specifications.

Analytical data for three representative, unstandardised batches of the protein-glutamine glutaminase enzyme preparation are shown in (**Table 1**). These data show compliance with the purity criteria of the specification.

Table 1. Analytical data for three representative enzyme product (unstandardised) batches

Control parameter	Unit	Specification	Batch 1	Batch 2	Batch 3
Protein-glutamine glutaminase activity	PGLU-A/g		148000	121000	102000
Lead	mg/kg	≤ 5	ND (LOQ < 0.5)	ND (LOQ < 0.5)	ND (LOQ < 0.5)
Arsenic	mg/kg	≤ 3	ND (LOQ < 0.3)	ND (LOQ < 0.3)	ND (LOQ < 0.3)
Cadmium	mg/kg	≤ 0.5	ND (LOQ < 0.05)	ND (LOQ < 0.05)	ND (LOQ < 0.05)
Mercury	mg/kg	≤ 0.5	ND (LOQ < 0.05)	ND (LOQ < 0.05)	ND (LOQ < 0.05)
Total viable count	CFU/g	≤ 10000	100	<100	<100
Total coliforms	CFU/g	≤ 30	<4	<4	<4
Enteropathogenic *Escherichia coli*	CFU/25 g	ND	ND	ND	ND
Salmonella spp.	CFU/25 g	ND	ND	ND	ND
Antimicrobial activity	—	ND	ND	ND	ND
Production strain	CFU/g	ND	ND	ND	ND

ND: not detected; LOQ: limit of quantification; CFU: colony forming unit

A certificate of analysis of the presented data is attached as **Appendix 3.1**. The methods of analysis used to determine compliance with the specifications are enclosed (**Appendix 3**).

The protein-glutamine glutaminase enzyme preparation is available as a liquid enzyme product. The concentrate is standardised in protein-glutamine glutaminase units (PGLU-A/g; **Appendix 3.2**). The preparation does not contain known food allergens (**Appendix 2.1** and **Appendix 4.3**).

A.6 Analytical method for detection

The protein-glutamine glutaminase enzyme preparation is to be used in the food industry as a processing aid. This information is not required in the case of an enzymatic processing aid.

B Information related to the safety of a chemical processing aid

Not applicable – this application does not concern a chemical processing aid.

C Information related to the safety of an enzyme processing aid

C.1 General information on the use of the enzyme as a food processing aid in other countries

The enzyme is used as processing aid during processing of peptide-bound L-glutamine-containing raw materials in a range of countries, where there are no restrictions of the use of enzyme processing aids or where the enzyme is covered by a country positive list or specific approval.

The safety of the protein-glutamine glutaminase preparation has been evaluated and confirmed by external expert groups, as follows:

- Denmark: The enzyme preparation was safety assessed resulting in the authorisation of the enzyme product by the Danish Veterinary and Food Administration.

C.2 Information on the potential toxicity of the enzyme processing aid

(a) Information on the enzyme's prior history of human consumption and/or its similarity to proteins with a history of safe human consumption

A wide variety of enzymes are used in food processing. Enzymes, including protein-glutamine glutaminase, have a long history of use in food (Pariza and Foster, 1983; Pariza and Johnson, 2001).

A protein-glutamine glutaminase enzyme preparation is widely authorised in, e.g. Australia and New Zealand, Canada, and China.

(b) Information on any significant similarity between the amino acid sequence of the enzyme and that of known protein toxins

A sequence homology assessment of the protein-glutamine glutaminase enzyme to known toxins was conducted. The amino acid sequence of the protein-glutamine glutaminase provided in **Appendix 6.5** was used as input for the search. No structural homologies to known toxins were found. The complete search report is enclosed in **Appendix 5**.

The use of substitute toxicological data from closely related production strains for safety assessment has been recognized by EFSA (EFSA, 2021) and by JECFA in their Principles and Methods for the risk assessment of chemicals in food, Subchapter 9.1.4.2 on Enzymes (JECFA, 2020). Following the principles, instead of the toxicological testing on the enzyme subject of this petition, the substitute toxicological data obtained from strains closely related to the production strain subject of this petition is referred to as the safety data. This protein-glutamine glutaminase production strain and another strain for the production of transglutaminase are closely related in the same safe strain lineage (**Appendix 6**). Therefore,

the previously conducted safety studies on the transglutaminase production strain can be used as a substitute to show the safety of this protein-glutamine glutaminase production strain. The toxicological testing of the transglutaminase was conducted on batches of transglutaminase concentrate (PPN66927 and PPN81685). The tox test batch is a transglutaminase enzyme concentrate without any addition of additives or other standardisation or stabilisation ingredients.

The safety studies as described below were performed on a representative batch (PPN66927 and PPN81685) that was produced according to the description given in section 3.3.2 A.4, omitting stabilization and standardization. As a result, batch (PPN66927 and PPN81685 represents the enzyme concentrate and not the final enzyme product.

A summary of the safety studies is enclosed in **Appendix 5.2**.

The following studies were performed:

- Ames Test. Test for mutagenic activity (**Appendix 5.3**)
- *In vitro* micronucleus test (**Appendix 5.4**)
- Toxicity study by oral gavage administration to rats for 13 weeks (**Appendix 5.5**)

The main conclusions of the safety studies can be summarised as follows:

- Transglutaminase PPN66927 and PPN81685 did not induce gene mutations in bacteria either in the presence or absence of metabolic activation (S-9) when tested under the conditions employed in this study.
- Transglutaminase PPN66927 and PPN81685 did not induce micronuclei in cultured human peripheral blood lymphocytes following treatment in the presence or absence of an aroclor induced rat liver metabolic activation system (S-9).
- Oral gavage administration of Transglutaminase PPN66927 and PPN81685 to rats at doses up to 100 % of the tox test batch (and 372 mg TOS/kg bw/day for 13 weeks was well-tolerated and did not cause any adverse change. The NOAEL was considered to be 100 % of the tox test batch (equivalent to and 372 mg TOS/kg bw/day).

Based on the toxicity data, it can be concluded that the transglutaminase enzyme preparation, represented by batch PPN66927 and PPN81685, exhibits no toxicological effects under the experimental conditions described.

C.3 Information on the potential allergenicity of the enzyme processing aid

(a) Information of the source of the enzyme processing aid

The protein-glutamine glutaminase enzyme is produced by a *Bacillus licheniformis* microorganism expressing the protein-glutamine glutaminase from *Chryseobacterium viscerum*. *Bacillus licheniformis* is ubiquitous in the environment and in general considered as a non-pathogenic microorganism (see Section 3.3.2 D).

(b) Analysis of similarity between the amino acid sequence of the enzyme and that of known allergens

Enzymes have a long history of safe use in food, with no indication of adverse effects or reactions. Moreover, a wide variety of enzyme classes (and structures) are naturally present in food.

The allergenicity potential of enzymes was studied by Bindslev-Jensen et al. (2006) and reported in the publication: “Investigation on possible allergenicity of 19 different commercial enzymes used in the food industry”. The investigation comprised enzymes produced by wild-type and genetically modified strains as well as wild-type enzymes and protein engineered variants and comprised 400 patients with a diagnosed allergy to inhalation allergens, food allergens, bee or wasp. It was concluded from this study that ingestion of food enzymes in general is not likely to be a concern with regard to food allergy.

Additionally, food enzymes are used in small amounts during food processing resulting in very small amounts of the enzyme protein in the final food. A high concentration generally equals a higher risk of sensitization, whereas a low level in the final food equals a lower risk (Goodman et al., 2008).

A sequence homology assessment of the protein-glutamine glutaminase enzyme to known allergens was conducted (**Appendix 5**). The amino acid sequence of the protein-glutamine glutaminase provided in **Appendix 6.5** was used as input for the search. The protein-glutamine glutaminase was compared to allergens from the COMprehensive Protein Allergen REsource (COMPARE: https://comparedatabase.org).

The analyses of the protein-glutamine glutaminase’s sequence identified no matches above threshold to allergens present in the databases.

On the basis of the available evidence, it is concluded that oral intake of the protein-glutamine glutaminase is not anticipated to pose any food allergenic concern.

C.4 Safety assessment reports prepared by international agencies or other national government agencies, if available

Documentation of approval of the protein-glutamine glutaminase in Denmark is enclosed in **Appendix 2**.

D Additional information related to the safety of an enzyme processing aid derived from a microorganism

D.1 Information on the source microorganism

The protein-glutamine glutaminase enzyme is produced by a *Bacillus licheniformis* microorganism expressing the protein-glutamine glutaminase from *Chryseobacterium viscerum*. The *Bacillus licheniformis* host strain Si3 was developed from the natural isolate *Bacillus licheniformis* Ca63. The Si3 strain lineage has a long history of safe use at Novozymes for production of food enzymes and has given rise to a number of food enzyme production

strains, which are used for production of previously evaluated and regulatory approved food enzymes.

The protein-glutamine glutaminase production strain is a non-pathogenic, non-toxigenic, genetically modified *Bacillus licheniformis* strain. The production strain is marker-free, and it does not produce secondary metabolites of toxicological concern to humans as explained in Section E 1.3, Section A.5 and **Appendix 6.1**.

D.2 Information on the pathogenicity and toxicity of the source microorganism

Industrial strains belonging to the *Bacillus licheniformis* species have a long history of safe use in food enzyme manufacturing (OECD, 1986). They have been used for decades in the production of enzymes, and in more than a decade as recombinant organisms for the production of a variety of bio-industrial products like food grade enzymes, vitamins, antibiotics, and additives (Schallmey et al., 2004).

The industrial production of alpha-amylase from *Bacillus licheniformis* was introduced in 1973 (Madsen et al., 1973). Since then, the bacterium has been safely used as a source of food enzymes such as carbohydrase (alpha-amylase) and protease for the production of various types of foods and food ingredients.

The Scientific Committee of EFSA has proposed to include a number of *Bacillus* species, including *Bacillus licheniformis*, on the list of QPS (Qualified Presumption of Safety) microorganisms due to the substantial body of knowledge available about these bacteria. Since all bacteria within the listed species potentially possess toxigenic traits, absence of toxigenic activity (emetic food poisoning toxins with surfactant activity and enterotoxic activity) must be verified (EFSA, 2007).

The Food and Drug Administration has affirmed that mixed carbohydrase and protease enzyme products derived from *Bacillus licheniformis* are generally recognized as safe (GRAS) in the production of certain foods including nutritive sweeteners, see 21CFR §184.1027 (Food and Drug Administration (FDA), 1983). In the supplementary information to the final rule in the Federal Register, FDA emphasized that “Published scientific literature as well as standard books on food microbiology demonstrate that *B. licheniformis* is widely recognized as a common contaminant found in many foods. None of these references report any toxicity or pathogenicity associated with the presence of this organism in food.”

In addition, the FDA did not question the conclusion that various other food enzymes obtained from genetically modified *Bacillus licheniformis* strains are GRAS under the intended conditions of use (GRN no. 22, 24, 72, 79, 265, 277, 472, 564, 572, 587, 594, 645, 689, 728, and 774).

JECFA has evaluated food enzymes derived from *Bacillus licheniformis*, including a genetically modified strain, and concluded that these food enzymes do not constitute a toxicological hazard (JECFA, 1987; JECFA, 2003).

The non-pathogenicity and non-toxigenicy of *Bacillus licheniformis* are thus strongly supported by the historic record of this organism.

The genetically modified *Bacillus licheniformis* host strain, used for production of the enzyme product, has been developed in a line of strains which have been used for production at Novozymes for many years and has an extensive history of commercial safe use for production of recombinant enzymes for food. It has been constructed using only well-characterized genetic material from class 1 microorganisms. No genetic material that can give rise to resistance towards antibiotics was left in the recipient strain as a result of the genetic modifications.

D.3 Information on the genetic stability of the source organism

The inserted recombinant DNA is genetically stable during fermentation, as the inserted DNA is integrated into the chromosome.

Stability of the introduced DNA sequences was analysed using phenotypic characteristics of the production strain, *i.e.* enzyme activity and protein synthesis (**Appendix 6.6**).

For a more detailed description of the strain construction and characteristics, please see section 3.3.2 E.

E Additional information related to the safety of an enzyme processing aid derived from a genetically-modified microorganism

E.1 Information on the methods used in the genetic modification of the source organism

This section contains summarised information on the modifications of the host strain, on the content and nature of the introduced DNA and on the construction of the final production strain, as well as the stability of the inserted gene. The detailed information is provided in the confidential **Appendix 6**.

E.1.1 Host organism

The production strain was developed from the *Bacillus licheniformis* Si3 cell lineage, which was derived from the natural isolate *Bacillus licheniformis* Ca63. The Si3 cell lineage has a long history of safe use at Novozymes for production of food enzymes and has given rise to a number of food enzyme production strains, which are used for production of previously evaluated and regulatory approved food enzymes. The taxonomic classification of the recipient strain is:

Phylum	Firmicutes
Class	Bacilli
Order	Bacillales
Family	Bacillaceae
Genus	*Bacillus*
Species	*Bacillus licheniformis*

The recipient strain used in the construction of the *Bacillus licheniformis* production strain, was derived from the parental strain through a combination of classical mutagenesis/selection and GM-steps. These steps were carried out in order to simplify purification, enhance product stability and increase the safety of the strain.

E.1.2 Introduced DNA

The vector used to transform the *Bacillus licheniformis* recipient strain are based on *Staphulococcus aureaus* standard vectors (Gryczan et al., 1978; Horinouchi and Weisblum, 1982). No elements of the vector are left in the production strain. The protein-glutamine glutaminase expression cassette consisting of a hybrid *Bacillus* promoter, the coding sequence for protein-glutamine glutaminase from *Chryseobacterium viscerum* and a *Bacillus* terminator. The inserted protein-glutamine glutaminase gene was provided as a synthetic gene.

E.1.3 Construction of the recombinant microorganism

The *Bacillus licheniformis* production strain was constructed from the recipient strain through the following steps:

1. The protein-glutamine glutaminase expression cassette was integrated at specific integration sites present in the recipient strain.
2. A transformant was screened for rapid growth and high protein-glutamine glutaminase activity leading to the final production strain.

E.1.4 Antibiotic resistance gene

No functional antibiotic resistance genes were left in the strain as a result of the genetic modifications. Further details can be found in **Appendix 6.1**

E.1.5 Stability of the introduced genetic sequences

The transforming DNA is stably integrated into the *Bacillus licheniformis* chromosome and, as such, is poorly mobilised for genetic transfer to other organisms and is mitotically stable. Stability of the introduced DNA sequence was analysed using phenotypic characteristics of the production strain, *i.e.* enzyme activity and protein synthesis. Further details can be found in **Appendix 6.6**.

F Information related to the dietary exposure to the processing aid

F.1 A list of foods or food groups likely to contain the processing aid or its metabolites

The protein-glutamine glutaminase preparation is used as a processing aid during the processing of raw materials containing peptide-bound L-glutamine. In the presence of water, a protein-glutamine glutaminase catalyses deamidation of gamma-amide of peptide-bound L-glutamine to form peptide-bound L-glutamate with subsequent release of ammonia.

F.2 The levels of residues of the processing aid or its metabolites for each food or food group

The protein-glutamine glutaminase enzyme preparation is used in the following food manufacturing processes:

- Production of heat-treated milk and milk-based barista products
- Production of cheese
- Production of fermented milk products
- Production of modified milk protein
- Production of plant-based analogues of milk and milk products
- Production of protein hydrolysates from plants and fungi
- Processing of yeast and yeast products
- Production of protein hydrolysates from meat and fish proteins
- Production of plant-based meat analogues

Use level

The enzyme preparation is used at minimum levels necessary to achieve the desired effect and according to requirements for normal production following GMP.

The conditions of use of the protein-glutamine glutaminase preparation during food processing do not only depend on the type of application, but also on the food production process of each individual food manufacturer. In order to ensure optimal effectiveness of the enzyme at an acceptable economic cost the dosage, reaction time, process conditions and processing steps are adjusted.

The highest dosage given for solid food is 165 mg TOS/ kg raw material (plant and fungal proteins).

The highest dosage given for liquids is to 66 mg TOS/ kg raw material (plant-based raw materials).

Enzyme residues in the final food

The properties of use of the food enzyme are technologically beneficial in various industrial food manufacturing processes, e.g. improving the processing of protein, increase protein solubility, emulsifying and foaming properties, and improving their flavour-enhancing properties (Liu et al., 2022).

The food enzyme object of the present dossier is intended for use as a processing aid in food manufacturing processes, i.e. in production of heat-treated milk and milk-based barista products, cheese, fermented milk products, modified milk protein, plant-based analogues of milk and milk products, protein hydrolysates from plants and fungi, yeast and yeast products, protein hydrolysates from meat and fish proteins, plant-based meat analogues.

These food manufacturing processes include processing steps where the food enzyme is denatured, and therefore the food enzyme does not perform any technological function in the final food.

The enzyme protein itself is similar to other proteins in food and is not expected to have any significant interaction with food components. The reaction products (deamidated proteins and peptides) are normal constituents of the human diet.

In conclusion, the food enzyme does not exert any (unintentional) enzymatic activity, and it does not create any safety-related undesirable effects during its use in food processing.

F.2.1 Estimates of human consumption.

Method used for the dietary exposure assessment

An exposure assessment according to the Budget Method (Douglass et al., 1997; Hansen, 1966; ILSI, 1997) has been performed as the enzyme is used as a processing aid in various food manufacturing processes.

Budget Method

Overall, the human exposure to the protein-glutamine glutaminase will be negligible because the enzyme preparation is used as a processing aid and in low dosages.

The Budget Method assumptions represent a “maximum worst case” situation of human consumption, in which the food enzyme object of the present application would be used at its maximum recommended dosages in all processed food and all processed beverages and not only in those food and drink processes described in Section F.2.

It is also supposed that the totality of the food enzyme will end up in the final food. This assumption is exaggerated since the enzyme protein and the other substances resulting from the fermentation are diluted or removed in certain processing steps.

Therefore, the safety margin calculation derived from this method is highly conservative.

Assumptions in the Budget Method

Solids	The maximum energy intake over the course of a lifetime is 50 kcal/kg body weight/day.
	50 kcal corresponds to 25 g foods.
	Therefore, adults ingest 25 g foods per kg body weight per day.
	Assuming that 50 % of the food is processed food, the daily consumption will be 12.5 g processed foods per kg body weight.
	It is further assumed that all processed products contain a maximum of 20.0% protein.
Liquids	The maximum intake of liquids (other than milk) is 100 ml/kg body weight/day.
	Assuming that 25 % of the non-milk beverages is processed, the daily consumption will be 25 ml processed beverages per kg body weight.
	It is assumed that the densities of the beverages are ~ 1.
	It is further assumed that all processed beverages contain a maximum of 20% of cereal ingredient.

TMDI (Total amount of dietary intake) calculation

Solid food

The maximum recommended dosage given in Section 5.3 for the production of protein hydrolysates from plants and fungi and plant-based meat dialogues is up to 165 mg TOS/ kg raw material (plant and fungal proteins).

Based on this, 12.5 gram processed solid food per kg bw will maximally contain:

165 mg TOS per kg / 1000 g per kg x 12.5 g x 0.20 (20% protein) = 0.41 mg TOS

Liquids

The maximum recommended dosage given in Section 5.3 for production of modified milk protein is up to 66 mg TOS/ kg raw material (plant-based raw materials).

Based on this, 25 mL processed liquid will maximally contain:

66 mg TOS per kg / 1000 g per kg x 25 g x 0.20 (20% cereal ingredient) = 0.33 mg TOS

The Theoretical Maximum Daily Intake (TMDI) of consumers of the food enzyme is:

0.41 + 0.33 = 0.74 mg TOS/kg bw/day

F.2.2. Safety Margin Calculation

The safety margin is calculated as dose level with no adverse effect (NOAEL) divided by the estimated human consumption (TMDI). The NOAEL dose level in the 13 weeks oral toxicity study in rats was concluded to be 372 mg TOS/kg bw/day (cf. Section 3.3.2 C 2).

The estimated human consumption is 0.74 mg TOS/kg/day

The safety margin can thus be calculated to be 372/0.74 ≈ 508.

F.3 For foods or food groups not currently listed in the most recent Australian or New Zealand National Nutrition Surveys (NNSs), information on the likely level of consumption

Not relevant.

F.4 The percentage of the food group in which the processing aid is likely to be found or the percentage of the market likely to use the processing aid

It is assumed that all raw materials containing peptide-bound L-glutamine are processed using the protein-glutamine glutaminase object of this submission as a processing aid at the highest recommended dosage.

F.5 Information relating to the levels of residues in foods in other countries

As described in F.2.1 above, a “worst case” calculation is made assuming that all organic matter originating from the enzyme is retained in the processed food product. The dietary exposure is estimated using the Budget Method as the enzyme is used as a processing aid in various food manufacturing processes.

F.6 For foods where consumption has changed in recent years, information on likely current food consumption

No significant changes in recent years are observed.

List of references

Aunstrup K (1979) Production, isolation, and economics of extracellular enzymes. In: *Applied Biochemistry and Bioengineering*. Elsevier, pp. 27–69.

Bindslev-Jensen C, Skov PS, Roggen EL, Hvass P and Brinch DS (2006) Investigation on possible allergenicity of 19 different commercial enzymes used in the food industry. *Food and Chemical Toxicology* 44(11). Elsevier: 1909–1915.

Douglass JS, Barraj LM, Tennant DR, Long WR and Chaisson CF (1997) Evaluation of the budget method for screening food additive intakes. *Food Additives & Contaminants* 14(8). Taylor & Francis: 791–802.

EFSA (2007) Introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA-Opinion of the Scientific Committee. *EFSA Journal* 5(12). Wiley Online Library: 587.

EFSA CEP Panel. (2021). Scientific Guidance for the submission of dossiers on Food Enzymes. EFSA Journal, 19(10), 6851, 37 pp. https://doi.org/10.2903/j.efsa.2021.6851

FAO/WHO. (2006). General Specifications and Considerations for Enzyme Preparations Used in Food Processing. *Compendium of Food Additive Specifications, Sixty-Seventh Meeting. FAO JECFA Monographs 3*, 63–67. https://openknowledge.fao.org/server/api/core/bitstreams/f3a3df7e-7407-487e-a0b9-306f6a5e6828/content

Food and Drug Administration (FDA) (1983) Direct Food Substances Affirmed as GRAS, Mixed Carbohydrase and Protease Enzyme Product. 21 CFR Part 184. *Federal Register* 51(75): 13293.

Goodman RE, Vieths S, Sampson HA, Hill D, Ebisawa M, Taylor SL and Van Ree R (2008) Allergenicity assessment of genetically modified crops–what makes sense? *Nature biotechnology* 26(1). Nature Publishing Group: 73–81.

Gryczan TJ, Contente S and Dubnau D (1978) Characterization of Staphylococcus aureus plasmids introduced by transformation into Bacillus subtilis. *Journal of Bacteriology* 134(1). Am Soc Microbiol: 318–329.

Hansen SC (1966) Acceptable daily intake of food additives and ceiling on levels of use. *Food and cosmetics toxicology* 4. Elsevier: 427–432.

Horinouchi S and Weisblum B (1982) Nucleotide sequence and functional map of pE194, a plasmid that specifies inducible resistance to macrolide, lincosamide, and streptogramin type B antibodies. *Journal of Bacteriology* 150(2). Am Soc Microbiol: 804–814.

ILSI (1997) An evaluation of the budget method for screening food additive intake. Summary report of an ILSI Europe food chemical intake task force. ILSI Europe, Brussels, Belgium.

JECFA (1987) *Carbohydrase (alpha-amylase) from Bacillus licheniformis*. World Health Organization. Available at: https://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=1957.

JECFA (2003) Alpha-amylase (thermostable) from Bacillus licheniformis containing a modified alpha-amylase gene from B. licheniformis. World Health Organization. Available at: https://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=4382.

JECFA. (2020) Subchapter 9.1.4.2 of Principles and Methods for the Risk Assessment of Chemicals in Food. Environmental Health Criteria, 240.

Liu, X., Wang, C., Zhang, X., Zhang, G., Zhou, J., & Chen, J. (2022). Application Prospect of Protein-Glutaminase in the Development of Plant-Based Protein Foods. Foods, 11(3), Article 3. https://doi.org/10.3390/foods11030440.

Madsen GB, Norman BE and Slott S (1973) A new, heat stable bacterial amylase and its use in high temperature liquefaction. *Starch-Stärke* 25(9). Wiley Online Library: 304–308.

OECD (1986) *Recombinant DNA Safety Considerations: Safety Considerations for Industrial, Agricultural, and Environmental Applications of Organisms Derived by Recombinant DNA Techniques*. Organization for Economic Co-operation; Development.

Pariza MW and Foster EM (1983) Determining the safety of enzymes used in food processing. *Journal of Food Protection* 46(5). International Association for Food Protection: 453–468.

Pariza MW and Johnson EA (2001) Evaluating the safety of microbial enzyme preparations used in food processing: Update for a new century. *Regulatory Toxicology and Pharmacology* 33(2). Elsevier: 173–186.

Schallmey M, Singh A and Ward OP (2004) Developments in the use of Bacillus species for industrial production. *Canadian journal of microbiology* 50(1). NRC Research Press Ottawa, Canada: 1–17.

List of appendices

Appendices listed in **red, bold** font should be treated as confidential commercial information.

Appendix 1.1 Formal request for treatment of confidential commercial information (CCI)

Appendix 1.2 Checklist for general requirements

Appendix 1.3 Checklist for applications for substances added to food

Appendix 2.1 Product data sheet

Appendix 2.2 Danish approval document

Appendix 3.1 Certificate of analysis

Appendix 3.2 CCI Method to determine Protein-glutamine glutaminase activity

Appendix 3.2 non-CCI Method to determine Protein-glutamine glutaminase activity

Appendix 3.3 Method to determine heavy metals

Appendix 3.4 Method to determine total viable count

Appendix 3.5 Method to determine total coliforms

Appendix 3.6 Method to determine *Escherichia coli*

Appendix 3.7 Method to determine *Salmonella* spp.

Appendix 3.8 Method to determine antimicrobial activity

Appendix 3.9 CCI Absence of viable cells of the production strain in the food enzyme

Appendix 3.9 non-CCI Absence of viable cells of the production strain in the food enzyme

Appendix 4.1 GMP statement

Appendix 4.2 ISO certificate

Appendix 4.3 CCI Raw materials used during fermentation and recovery

Appendix 4.3 non-CCI Raw Materials used during fermentation and recovery

Appendix 5.1 Sequence homology assessment

Appendix 5.2 Summary of toxicity data

Appendix 5.3 Ames test report

Appendix 5.4 *In vitro* micronucleus study

Appendix 5.5 Toxicity study by oral gavage administration to rats for 13 weeks

Appendix 6 CCI Documentation regarding the production strain

Appendix 6 non-CCI Documentation regarding the production strain