

**27 October 2020**

**[139-20]**

**Supporting document 1**

Risk and Technical assessment – Application A1204

Βeta-amylase from soybean (Glycine max) as a processing aid (enzyme)

# Executive summary

The purpose of the application is to amend Schedule 18 – Processing Aids of the Australia New Zealand Food Standards Code (the Code) to include the enzyme beta-amylase (β-amylase) (EC 3.2.1.2) produced from soybeans (*Glycine max*).β-Amylase is proposed as a processing aid in starch processing for the production of maltose syrup.

The evidence presented to support the proposed use of the enzyme provides adequate assurance that the enzyme, in the quantity and form proposed to be used, is technologically justified and has been demonstrated to be effective in achieving its stated purpose. The enzyme meets international identity and purity specifications.

β-Amylase from soybean is derived from the edible parts of the *Glycine max* plant, for which a history of safe use over generations is well known.

FSANZ considers that soybean β-amylase is unlikely to pose an allergenicity concern. Bioinformatic analysis identified a degree of amino acid sequence homology between β-amylase from soybean and an allergenic protein from wheat, but FSANZ does not consider β-amylase to be of allergenic concern in wheat allergic individuals given the likely very low exposure and that the enzyme is likely to be digested in the stomach like other dietary proteins.

The WHO/IUIS Allergen Nomenclature Database lists seven soy proteins that are food allergens. β-amylase from soybean is not one of these seven allergenic soy proteins and is not an allergen to individuals with soybean food allergy. However, as the enzyme is derived from soy it is possible that the enzyme preparation may contain traces of these allergenic proteins due to carry over from the production process.

Based on the available evidence there are no safety concerns from the proposed uses of β-amylase from soy as a processing aid. Given the long history of safe use of soy and soy products and the absence of an identifiable hazard from the enzyme, an acceptable daily intake (ADI) ‘not specified’ is appropriate. A dietary exposure assessment was therefore not required.

Table of contents

[Executive summary i](#_Toc54185712)

[1 Introduction 2](#_Toc54185713)

[**1.1** **Objectives of the assessment** 2](#_Toc54185714)

[2 Food technology assessment 2](#_Toc54185715)

[2.1 Characterisation of the enzyme 2](#_Toc54185716)

[2.1.1 Identity of the enzyme 2](#_Toc54185717)

[2.1.2 Technological purpose of the processing aid 3](#_Toc54185718)

[2.1.3 Technological justification for the enzyme 4](#_Toc54185719)

[2.2 Manufacturing process 4](#_Toc54185720)

[2.2.1 Production of the enzyme 4](#_Toc54185721)

[2.2.2 Allergen considerations 5](#_Toc54185722)

[2.2.3 Specifications 5](#_Toc54185723)

[2.3 Food technology conclusion 6](#_Toc54185724)

[3 Safety of the enzyme 6](#_Toc54185725)

[3.1 History of use 6](#_Toc54185726)

[3.2 Potential toxicity of the processing aid 7](#_Toc54185727)

[3.3 Potential for allergenicity 7](#_Toc54185728)

[3.4 Assessments by other regulatory agencies 8](#_Toc54185729)

[4 Discussion 9](#_Toc54185730)

[5 Conclusion 9](#_Toc54185731)

[6 References 9](#_Toc54185732)

# 1 Introduction

Danisco New Zealand Ltd (Danisco) applied to FSANZ for permission to use the enzyme beta-amylase (β-amylase) (EC 3.2.1.2) as a processing aid in starch processing for the production of maltose syrup. This β-amylase is a biological isolate produced from soybeans (*Glycine max*).

There are permissions for β-amylase from two other plant sources in the Code (sweet potato and malted cereals), however, not when produced from soybean. There is also permission for β-amylase produced from two microorganisms. If permitted following a pre-market assessment, Danisco’s β-amylase will provide an additional option for manufacturers of maltose syrup.

**1.1 Objectives of the assessment**

The objectives of this risk and technical assessment were to:

* determine whether the proposed purpose is clearly stated and that the enzyme achieves its technological function in the quantity and form proposed to be used as a food processing aid
* evaluate potential public health and safety concerns that may arise from the use of this enzyme, as a processing aid. Specifically by considering the:
* history of use of the source – soybean, and
* safety of the enzyme.

# 2 Food technology assessment

## Characterisation of the enzyme

### Identity of the enzyme

The applicant provided relevant information regarding the identity of the enzyme, and this has been verified using an appropriate enzyme nomenclature reference (IUBMB 2020).

Accepted IUBMB[[1]](#footnote-2) name: β-amylase [[2]](#footnote-3)

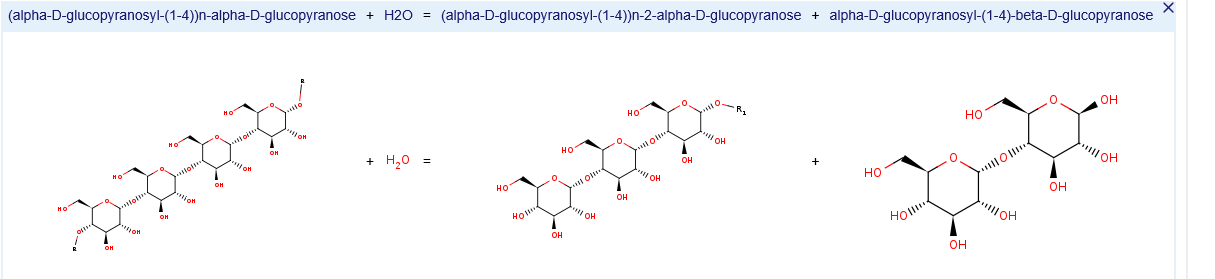
Systematic name: 4-α-D-glucan maltohydrolase

Other names: saccharogen amylase, glycogenase; 1,4-α-D-glucan maltohydrolase

IUBMB enzyme nomenclature: EC 3.2.1.2

CAS[[3]](#footnote-4) number: 9000-91-3

Reaction: hydrolysis of (1→4)-α-D-glucosidic linkages in polysaccharides so as to remove successive maltose units from the non-reducing ends of the chains (Figure 1).



SOURCE: BRENDA:EC 3.2.1.2 (<https://www.brenda-enzymes.org/enzyme.php?ecno=3.2.1.2>)

***Figure 1*** *Representation of hydrolysis reaction of a polysaccharide catalysed by* β–amylase

### Technological purpose of the processing aid

The applicant states that the technological purpose of β-amylase in starch processing is as an exoamylase that catalyses the release of successive maltose units by hydrolysing 1,4-alpha-D-glucosidic linkages from the non-reducing end of the dextrin chain. The applicant states β-amylase can be used in conjunction with pullulanase (a debranching enzyme hydrolyzing 1,6-α-D-glucosidic bonds), to generate more substrates for β-amylase to hydrolyse, therefore leading to higher maltose contents. Combined usage of both β-amylase and pullulanase is typical during very-high maltose (maltose >65%) and ultra-high maltose (maltose >80%) production.

The stated technological purpose, and use in conjunction with pullulanase is supported by scientific literature (Damodaran et al, 2008, Nagodawithana and Reed,1993).

Danisco provided information on the physical and chemical properties of the enzyme preparation. Table 1 summarises this information.

***Table 1*** β -amylase enzyme preparation physical/chemical properties

|  |  |
| --- | --- |
| **Physical/chemical properties of commercial enzyme preparation** | |
| Enzyme activity | 25000-35000 SBAB /g (average from 3 batch results provided). \* |
| Appearance | Tan to brown liquid. |
| Temperature range | activity within range 30-76°C.  optimum 50-60°C. |
| Temperature stability | Retains over 80% of activity for 30min @ up to 60°C.  Almost no remaining activity at temperature ≥ 70°C. |
| pH range | Optimum around pH 6.0 with activity within range 3.8 – 8.0.  Residual activity reduced to around 40% at pH 3.0 for 2 hours. |
| Storage stability | approx. 95% remaining activity when stored for 12 months @ 4°C. |

\*Soybean Amylase Beta/g (SBAB/g). One SAB is defined as the amount of mg of maltose produced by 1 mg enzyme liquid (or 1 mg starch) in hydrolysing 1.10% starch per hour under the conditions of pH 5.50 and 60 °C.

Use of commercial enzyme preparations should follow good manufacturing practice (GMP), where use is at a level that is not higher than that necessary to achieve the desired enzymatic reaction. The conditions of use of the enzyme during maltose syrup production will depend on a number of factors including the nature of the application and the individual food manufacturers’ production processes. The optimum use level should be assessed and adjusted using trials that reflect their particular processes.

Table 2 below summarises indicative use levels of the applicant’s enzyme preparation. The average total organic solid (TOS) content of the enzyme preparation is 79.9%. The enzyme preparation will be used in maltose production at 0.15 - 1.15 kg enzyme product/metric ton dry material (MTDM)[[4]](#footnote-5). This is equivalent to a maximum of 919 mg TOS/kg dry material.

Table 2 Indicative use levels of β-amylase

|  |  |  |  |
| --- | --- | --- | --- |
| Application | Raw Material (RM) | Recommended usage levels (mg enzyme product/kg RM) | Maximum TOS level (mg TOS/kg RM) |
| maltose production | starch | 0.15- 1.15 | 919 |

\*To distinguish the proportion of the enzyme preparation derived from the source material and manufacturing process from that contributed by intentionally added formulation ingredients, the content of total organic solids (TOS) is calculated as follows: % TOS = 100 - (A + W + D) where: A = % ash, W = % water and D = % diluents and/or other formulation ingredients.

### Technological justification for the enzyme

As outlined above, the application states that the enzyme fulfils an important technological purpose and provides the starch processing industry with the following benefits and opportunities: conversion of liquefied starch into a maltose rich solution; a more specific reaction with less formation of side products compared to acid catalysed hydrolysis; and energy savings in production and less wastewater. Appendix A3 to the application provides information on the use of the enzyme in starch processing. The enzyme is added at the saccharification step. Maltose syrup imparts sweetness and flavour to food.

## Manufacturing process

### Production of the enzyme

As the enzyme is a biological isolate from edible soybean (*Glycine max*) the production involves a separation and concentration step, followed by purification and formulation steps. The soybean raw material is not produced from genetically modified soybeans. Appendix A6 to the application provides a summary of the manufacturing process.

The applicant states that all raw materials used in the separation process (extraction and concentration) for the β-amylase enzyme concentrate are standard ingredients used in the enzyme industry. All the raw materials are reported to conform to the specifications of the Food Chemical Codex, 6th edition (FCC 2008), except for those raw materials which do not appear in the FCC. For those not appearing in the FCC, Danisco has internal requirements in line with FCC requirements and has in place a supplier quality program. Danisco manufacture their β-amylase in accordance with food good manufacturing practice.

After separation, the liquid containing the enzyme is concentrated to reach the desired enzyme activity and/or to increase the enzyme activity to total organic solids (TOS) ratio. The production process produces an enzyme preparation, as the commercial product.

Full details on the manufacturing process, raw materials and ingredients used in the production of Danisco’s β-amylase enzyme preparation were provided as “Confidential Commercial Information”.

### Allergen considerations

As the β-amylase is produced from soybean, soy is declared as an allergen in the data sheet supplied with the β-amylase enzyme preparation (Appendix A9 to the application). The data sheet does not list the presence of any other known allergens. In starch processing, the applicant has indicated that it is expected that the enzyme will be removed during production and refining processes (denatured by heat or removed during carbon or ion exchange treatments).

### Specifications

There are international specifications for enzyme preparations used in the production of food. These have been established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in its Compendium of Food Additive Specifications and in the Food Chemicals Codex (FCC). These specifications are included in S3—2 of the Code and enzymes used as a processing aid must meet either of these specifications.

Dansico states that the β-amylase enzyme preparation meets the general requirements for enzyme preparations for both the Food Chemicals Codex (FCC 2008). and JECFA specifications for identity and purity (JECFA 2006).

The JECFA 2006 and FCC 2008 specifications are included in the primary sources listed in section S3—2 of Schedule 3 of the Code (noting these publications are contained in earlier monographs or editions). Schedule 3 of the Code also includes specifications for heavy metals (section S3—4) if they are not already detailed within specifications in sections S3—2 or S3—3.

Table 3 provides a comparison of the analysis of different batches of the β-amylase product with international specifications established by JECFA and Food Chemicals Codex, as well as those in the Code (as applicable). Based on these results, the enzyme preparation meets all relevant specifications for metals and the microbiological criteria. As noted above, Danisco states that the processing aid complies with the specifications for identity and purity set by FCC and JECFA. This is broader than the specifications for metals and microbiological limits.

*Table 3 Comparision of Danisco’s β-amylase compared to JECFA, Food Chemicals Codex, and Code specifications for enzymes*

| Analysis | | Danisco analysis[[5]](#footnote-6) | Specifications |  | | |
| --- | --- | --- | --- | --- | --- | --- |
| Danisco  specification | JECFA | Food Chemicals Codex | Australia New Zealand Food Standards Code  (section S3—4) |
| Lead (mg/kg) | <0.04, 0.04, <0.099 | | <5 | < 5 | < 5 | ≤2 |
| Cadmium (mg/kg) | | <0.006, <0.005 (x2) | <0.5 | - | - | ≤1 |
| Arsenic  (mg/kg) | | <0.05, <0.06, <0.07 | <3 |  |  | ≤1 |
| Mercury (mg/kg) | | <0.01 (x3) | <0.5 | - | - | ≤1 |
| Coliforms (cfu/g) | | <10, <30, <10 | <30 | ≤30 | ≤30 | - |
| *Salmonella* (in 25 g) | | Negative | Negative | Absent | Negative | - |
| *E. coli* (in 25 g) | | <3, <3, <3 | <3 | Absent | - | - |
|  | |  |  |  |  |  |

## Food technology conclusion

FSANZ concludes that the use of this β-amylase in maltose syrup production is clearly described in the application and is consistent with its typical function of producing maltose units from polysaccharides. The evidence presented to support its proposed use provides adequate assurance that the enzyme, in the quantity and form proposed to be used (which must be consistent with GMP controls and processes), is technologically justified and effective in achieving its stated purpose. The enzyme meets international identity and purity specifications.

# 3 Safety of the enzyme

## 3.1 History of use

The β-amylase assessed in the present application is produced from the whey of non-genetically modified soybean. Soybeans are used to produce a range of foods including soy sprouts, boiled soybeans (edamame), roasted soybeans, full fat soy flour, soybean oil, meat analogues, infant formula and traditional soy foods (miso, soy milk, soy sauce, tempeh and tofu). Soy protein products are also added to a number of meat, dairy, bakery and cereal products as protein extenders. Unprocessed (raw) soybeans are not suitable for food because they contain anti-nutritional factors such as trypsin inhibitors and lectins, but adequate heat processing inactivates these anti-nutrients making them suitable for food use (OECD 2012).

β-Amylase from soy is a permitted food enzyme in China and β-amylase from the seeds of legumes is a permitted food additive in Japan. Several β-amylase enzymes from other microbial sources, malted cereals and sweet potato (*Ipomoea batatas*) are currently permitted as processing aids in Schedule 18 of the Code. The applicant conducted a search for homology between the β-amylase from *G. max* that is the subject of the present application and a selection of β-amylases from the species listed in Schedule 18 of the Code. The sequences of the β-amylases listed in Schedule 18 were retrieved from the UniProtKB database.

The highest homology between β-amylase from *G. max* and the analysed β-amylase enzymes was with β-amylase from wheat (69% identity).

## 3.2 Potential toxicity of the processing aid

β-Amylase is produced from the edible parts of a plant. Soybean and soy products are routinely eaten in many parts of the world (Erdman and Fordyce, 1989), providing a long history of safe use over generations.

As set out in the International Programme on Chemical Safety’s ‘Principles and Methods

for the Risk Assessment of Chemicals in Food’, enzymes obtained from edible portions of plants are regarded as foods and, consequently, considered acceptable provided satisfactory chemical and microbiological specifications can be established (FAO/WHO 2009). Toxicity or genotoxicity studies with β-amylase from non-GM *G. max* are therefore not required to support the safety of the enzyme preparation in this case.

## 3.3 Potential for allergenicity

Soybean is known to be one of the most common foods that can cause allergic reactions in Australia and New Zealand, and internationally. Risk management measures in the Code require labelling of foods containing soy.

Soybean β-amylase is not an allergen to individuals with soybean food allergy. No reports of food allergy to β-amylase from soy were identified in the scientific literature. Not all individuals who are allergic to wheat are also allergic to soy, and a study of the risk of food allergy among soy-allergic consumers consuming wheat contaminated with low levels of soy noted a lack of evidence of allergic reactions among soy-allergic consumers to wheat-based products (Remington et al. 2013).

The applicant performed several searches for homology of the amino acid sequence of the β-amylase enzyme to known allergens using the [Allergen Online database](http://www.allergenonline.org/) of the University of Nebraska’s Food Allergy Research and Resource Program (FARRP). The following searches were conducted:

* A search for full-length sequence alignment for matches of > 35% identity using an E value < 0.1
* A search using a sliding window of 80 amino acid stretches for identities > 35%
* A search for exact matches of 8 contiguous amino acids.

The search for full-length sequence alignment found one match with a peptide sequence of more than 35% identity to the mature soybean β-amylase sequence; a Chain A β-amylase(Tri a 17) in bread wheat (*Triticum aestivum*). Matches with this protein were also found with the other two searches. Chain A β-amylase from wheat is listed as a food allergen in the [WHO/IUIS Allergen Nomenclature Database](http://www.allergen.org/).

Given the sequence similarity between soy β-amylase and the wheat allergen, the applicant provided *in vitro* pepsin resistance and pancreatin resistance assays with β-amylase, summarised below.

#### In vitro pepsin resistance and pancreatin resistance of β-amylase (DuPont 2020) Regulatory status: Non-GLP, non-guideline

The test item in this study was the concentrated enzyme after aqueous extraction from *G. max*, diluted in water to a target concentration of 5.0 mg/mL.

In the pepsin resistance assay, β-amylase was incubated in simulated gastric fluid containing pepsin (SGF; pH ~1.2) at 37ºC for 0.5, 1, 2, 5, 10, 20, 30, and 60 minutes. The ratio of pepsin to β-amylase or control protein ratio was 10 units per microgram protein, resulting in a molar ratio equal to 3.5. Controls were SGF alone, bovine serum albumin (BSA) or β-lactoglobulin incubated in SGF for 0, 1, and 60 minutes and β-amylase in water or in gastric control solution without pepsin for 60 minutes.

In the pancreatin resistance assay, β-amylase was incubated in simulated intestinal fluid (SIF) containing pancreatin (SIF; pH ~7.5) for 0, 1, 2, 5, 10, 20, 30, 60, and 360 minutes. Controls were SIF alone, BSA or β-lactoglobulin incubated in SIF for 0, 1, and 60 minutes and β-amylase in water or in intestinal control fluid without pancreatin for 60 minutes. In both experiments samples were inactivated at the stated time points, subjected to SDS-PAGE and the resulting gels were stained with a protein staining agent.

β-Amylase was rapidly digested in SGF, with protein staining of the SDS-PAGE gel showing that the β-amylase band was no longer visible within 0.5 minutes of incubation in SGF. In contrast β-amylase was not digested in SIF after 360 minutes of incubation.

#### Conclusion on potential allergenicity

Soybean β-amylase is not an allergen to individuals with soybean food allergy. Bioinformatic analysis identified a degree of amino acid sequence homology between β-amylase from soy and an allergenic protein from wheat. FSANZ does not consider β-amylase to be of allergenic concern in wheat allergic individuals given the likely very low exposure and that the enzyme is likely to be digested in the stomach like other dietary proteins.

#### Carry-over of soybean allergens

The WHO/IUIS Allergen Nomenclature Database lists seven soy proteins that are food allergens. As the enzyme is derived from soy, the applicant has indicated in their allergen declaration that there is a possibility that the enzyme preparation may contain traces of these allergenic soy proteins due to carry over from the production process.

## 3.4 Assessments by other regulatory agencies

The European Food Safety Authority (EFSA) evaluated the safety of β-amylase from soybean in 2017. The proposed uses were maltose syrup production and the manufacture of a Japanese rice cake. EFSA concluded that β-amylase from soybean does not give rise to safety concerns under the intended conditions of use, with the exception that Japanese rice cake produced with the enzyme may contain traces of soybean allergens (EFSA 2017).

β-Amylase from soybean is also listed as a permitted food enzyme preparation in China[[6]](#footnote-7). Seeds of legumes are listed as a permitted source for beta-amylase in Japan’s [Specifications and Standards for Food Additives](http://www.nihs.go.jp/dfa/dfa-j/shokuten_kikaku_j.html).

# 4 Discussion

β-Amylase from soybean is derived from the edible parts of the *G. max* plant, for which a history of safe use over generations is well known. The enzyme also meets international identity and purity specifications.

FSANZ considers that soybean β-amylase is unlikely to pose an allergenicity concern. Bioinformatic analysis identified a degree of amino acid sequence homology between β-amylase from soybean and an allergenic protein from wheat, but FSANZ does not consider β-amylase to be of allergenic concern in wheat allergic individuals given the likely very low exposure and that the enzyme is likely to be digested in the stomach like other dietary proteins.

The WHO/IUIS Allergen Nomenclature Database lists seven soy proteins that are food allergens. β-amylase from soybean is not one of these seven allergenic soy proteins and is not an allergen to individuals with soybean food allergy. However, as the enzyme is derived from soy it is possible that the enzyme preparation may contain traces of these allergenic proteins due to carry over from the production process.

# 5 Conclusion

Based on the available evidence there are no safety concerns from the proposed uses of β-amylase from soy as a processing aid. Given the long history of safe use of soy and soy products and the absence of an identifiable hazard from the enzyme, an acceptable daily intake (ADI) ‘not specified’ is appropriate. A dietary exposure assessment was therefore not required.

# 6 References

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Remington BC, Taylor SL, Marx DB, Petersen BJ, Baumert JL (2013) Soy in wheat – contamination levels and food allergy risk assessment. Food Chem Toxicol 62:485-91.

1. International Union of Biochemistry and Molecular Biology. [↑](#footnote-ref-2)
2. The term that will be used in the proposed draft variation to the Code for this enzyme is β-Amylase EC 3.2.1.2, as this will ensure consistency with other existing permissions in Schedule 18 of the Code. [↑](#footnote-ref-3)
3. Chemical Abstracts Service. [↑](#footnote-ref-4)
4. Even though β-amylase hydrolyses the starch under liquid conditions, in the manufacturing process, the raw material is solid starch. The dry starch will be mixed with water and enzymes. The β-amylase use level is therefore calculated and reported in Table 2 based on dry material. [↑](#footnote-ref-5)
5. Three batches of Danisco enzyme preparation [↑](#footnote-ref-6)
6. National Standard of the People’s Republic of China, National Food Safety Standard for Uses of Food Additives. GB 2760-2015. [↑](#footnote-ref-7)