A Beta-amylase Enzyme
from Soybean (*Glycine max*)

PROCESSING AID APPLICATION

Food Standards Australia
New Zealand

Applicant: Danisco New Zealand Ltd

May 20, 2020

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EXECUTIVE SUMMARY:

DuPont Nutrition & Biosciences (N&B) is seeking approval for a "Beta-amylase (EC 3.2.1.2)" enzyme for use as processing aid in starching processing. The enzyme is designated as "Soy beta-amylase" or "SBA" throughout the dossier.

The enzyme SBA is a biological isolate from edible plant soybean (*Glycine max*). The source plant is not genetically modified.

The enzyme is intended for use in starching processing for manufacturing of maltose syrup. In the application, Beta-amylase converts starchy substrate to maltose. In the application, SBA will be used as a processing aid where the enzyme is not present in the final food.

To assess the safety of the SBA for use in these applications, DuPont vigorously applied the criteria identified in the guidelines as laid down by Food Standards Australia New Zealand (FSANZ) and U.S. Food and Drug Administration (FDA) utilising enzyme toxicology/safety data, the safe history of use of beta-amylase enzymes in food, the history of safe use of soybean in food, an allergenicity evaluation. As residual enzyme level and expected intake of enzyme in maltose syrup is negligible, no Margin of Safety was calculated.

Based on the results of safety evaluations, SBA has been demonstrated as safe for its intended applications and at the proposed usage levels. Approval of this application would provide manufacturers and/or consumers with benefits of facilitating the process of starch processing and lowering the manufacturing cost.

General information

1.1 Applicant details

(a) Applicant:

This application is made by Danisco New Zealand Ltd

(b) Company:

Dansico New Zealand Ltd

(c) [redacted]

(d) [redacted]

(e) Email Address:

See above

(f) Nature of Applicants Business:

Danisco New Zealand Ltd – A subsidiary of E. I. du Pont de Nemours and Company, manufacturer/marketer of specialty food ingredients, food additives and food processing aids.

(g) [redacted]

No other individuals, companies or organizations are associated with this application.

1.2 Purpose of the application

This application seeks to modify Schedule 18 to Standard 1.3.3 Processing Aids to permit the use of a new *Processing Aid*, subject of this application. The intended use of the processing aid is starching processing. SBA is added to the liquefied starch before the saccharification step, in order to convert liquefied starch into a maltose rich solution. This solution is purified and concentrated to a maltose syrup, to be used as a sweetener in a number of foodstuffs.

This application is made solely on behalf of DuPont Nutrition & Biosciences (N&B), the manufacturer/marketer of the *Processing Aid*. When approved, the *Processing Aid* would be available for use by any food manufacturer in Australia and New Zealand.

SBA, subject of this application, is intended for use in starching processing.

Currently no beta-amylase from soybean is permitted as a Processing Aid, however beta-amylase from malted cereals, sweet potato and other microbial sources are listed in Schedule 18 section S18-4(4,5) as permitted enzymes. Approval of this application would provide food processors with a new enzyme preparation offering the benefits and advantages as discussed in Section 2.3 and Appendix A.

1.3 Justification for the application

1.3.1. Regulatory Impact Information

A. Costs and Benefits of the application

SBA is an enzyme isolated from edible soybean. The source plant is non-genetically modified. The enzyme is characterised as a 4-α-D-glucan maltohydrolase (EC 3.2.1.2). A collection of information detailed in Section 3 supports the safety of the source organism and the enzyme for use in the applications outlined in Section 4.

SBA is intended for use in starching processing, where it converts starchy substrate to maltose. More information on the benefit of this enzyme can be found in Section 2.2 and Appendix A.

Enzyme preparations are widely used as processing aids in the manufacture of food products. Currently no beta-amylase from soybean is permitted as a Processing Aid. Approval of this application would provide food processors with a new enzyme preparation offering the benefits and advantages as discussed previously.

B. Impact on international trade

The inclusion of beta-amylase from soybean in the Australia New Zealand Food Standards Code as a processing aid may promote international trade on products produced with this enzyme product, and reduce technical barriers to trade.

1.4. Support for the application

No marketing or promotional activities have been undertaken for beta-amylase derived from soybean in the Australia/New Zealand market. Hence at this stage, no requests from food

manufacturers are provided in support of this application. However, the need and justification for use of the processing aid are discussed in Section 1.3, and it is anticipated that support from the food processing industry will be submitted during the period for public comment on the application Draft Regulatory Measure/Assessment Report.

1.5. Assessment Procedure

This application seeks to modify Schedule 18 to Standard 1.3.3 Processing Aids to permit the use of a Processing aid that is currently not permitted. Based on guidance in the Application Handbook, DuPont N&B considers General Procedure Level 1 (up to 350 hours) to be the appropriate procedure for assessment of the application.

1.6. Confidential Commercial Information (CCI)

Certain (identified) technical and manufacturing information included in 1.1 Applicant details, Appendices A6, Appendix B1 full allergenicity report and other information labelled with Confidential Commercial information is regarded by the applicant as **Confidential Commercial Information** and is provided in the application strictly on this basis. This information is the result of a significant research and development effort and investment by the applicant; it is not in the public domain and is considered as either proprietary or commercially sensitive. It would be disadvantageous to the applicant if this information were released into the public domain.

1.7. Exclusive Commercial Capturable Benefit (ECCB)

According to Section 8 of the FSANZ Act, this application is not expected to confer Exclusive Capturable Commercial Benefit (ECCB).

1.8. International and other National Standards

Refer to Appendix D for further details

1.8.1 Codex Standards

Beta-amylase from soybean has not been reviewed by JECFA.

1.8.2 International Legislation

Beta-amylase derived from soybean is approved in both China and Japan. Refer Appendix D.

1.9. Statutory declaration

I, [redacted]

of [redacted]

make the following declaration under the *Statutory Declarations Act 1959:*

1) The information provided in this application fully sets out the matters required

2) The information provided in this application is true to the best of my knowledge and belief

3) No information has been withheld which might prejudice this application, to the best of my knowledge and belief

I understand that a person who intentionally makes a false statement in a statutory declaration is guilty of an offence section 11 of the *Statutory Declarations Act 1959*, and I believe that the statements in this declaration are true in every particular.

Signature: [redacted]

Declared at [redacted] on 18th of May 2020

Before me [redacted]

1.10. Checklist

	Mandatory Requirements	Check	Page Number	Remarks
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3.3.2. Processing aids	A. Technical information on the processing aid	√	9	Section 2
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	D. Additional information related to the safety of an enzyme processing aid derived from a microorganism	×		Not applicable as SBA is not from microorganism
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2. Technical information

Please refer to Appendix A for further details

2.1. Type of processing aid

SBA enzyme is a biological isolate from edible plant soybean (*Glycine max*). The source plant is not genetically modified.

This Processing Aid falls into the category "Enzymes of plant origin" from the Food Standard Code section 1.3.3-6 Enzymes.

2.2. Identity

2.2.1 Chemical/Common Name:

According to IUBMB Enzyme Nomenclature, the systematic name of the principle enzyme activity is 4-α-D-glucan maltohydrolase. Other names used are saccharogen amylase; glycogenase; β amylase, β-amylase; 1,4-α-D-glucan maltohydrolase.

- EC number: 3.2.1.2
- CAS number: 9000-91-3

Biological source: SBA enzyme is a biological isolate from edible plant soybean (*Glycine max*). The source plant is not genetically modified.

2.2.2 Marketing Name of the Processing Aid:

The marketing name of this enzyme preparation will depend on the application. An example marketing name of SBA is OPTIMALT® SBA.

2.2.3 Molecular and Structural Formula:

SBA is a protein. The amino acid sequence is known. Please refer to Appendix B.

2.3. Chemical and physical properties

In principle, the enzymatic conversion of polysaccharides like starch with the help of SBA may be of benefit in the processing of all food raw materials which naturally contain the substrate. The benefits of the use of SBA in starch processing may include:

- Conversion of liquefied starch into maltose rich solution
- Compared to acid catalysed hydrolysis the reaction is more specific and there is less formation of side products
- Energy savings in production and less wastewater

Substrate specificity:

Beta-amylase uses solubilised starch as a substrate. The shortest normal saccharide that the enzyme is capable to hydrolyse is maltotetraose.

Activity:

The activity of the SBA 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 condition of pH 5.50 and 60 °C.

Temperature optimum:

Approximately between 50-60°C, activities observed from 30 to 76°C.

Thermal stability:

The enzyme activity dropped to below detection after incubation at above 70°C for 30 minutes.

pH optimum:

Approximately around pH 6.0, with activities observed from pH interval 3.8-8.0.

pH stability:

Enzyme activity is observed in the pH range 3.0-8.0 with 2 hours of incubation. Residual activity dropped to around 40% at pH 3.0 for 2 hours.

Interaction of the enzyme with different foods:

SBA enzyme preparation will be used as a processing aid where the enzyme is not present in the final food.

Nutritional implication:

SBA is a protein and any residual amounts remaining in food consumed would accordingly have the same nutritional value. However, the use levels of SBA are very low, and as with other enzymes that are currently approved and used as Processing Aids, use of this preparation would not have any nutritional significance.

2.4. Manufacturing process

SBA is produced under Quality Assurance Certification (ISO 9001) and Good Manufacturing Practices. Schematically, the production process of the β-amylase preparation comprises a separation and concentration step, followed by purification and formulation steps.

Full details on the raw materials used for the production are provided in Appendix A6. Note that this information is proprietary and "**Confidential Commercial Information**" status is requested.

The production of SBA is monitored and controlled by analytical and quality assurance procedures that ensure that the finished preparation complies with the specifications and is of the appropriate quality for use as a processing aid in food processing applications.

2.5. Specification for identity and purity

Impurity profile:

Appropriate GMP controls and processes are used in the manufacture of SBA to ensure that the finished preparation does not contain any impurities of a hazardous or toxic nature. The specification for impurities and microbial limits are as follows:

Metals:	
Lead	less than 5 mg/ kg
Arsenic	less than 1 mg/kg
Cadmium	less than 1 mg/kg
Mercury	less than 1 mg/kg
Microbiological:	
Total coliforms	less than 30 CFU/mL
E. coli	absent in 25 mL

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Salmonella	absent in 25 mL

Physical properties:

Appearance	Tan to brown liquid

Standard for identity:

SBA meets the specifications laid down by the Joint FAO/WHO Expert Committee on Food Additives and the Food Chemicals Codex.

3. Safety

Refer to Appendix B for further details

3.1. Use of the enzyme as a food processing aid in other countries

Enzyme products are developed for a specific function, i.e. to catalyse a specific chemical reaction. That reaction determines the IUBMB classification. Enzyme variants may be selected to have a better performance of that function under the specific conditions of the application (e.g. temperature or pH). Enzymes of a certain IUBMB classification share conserved structural elements, called domains, which are needed for their specific function. As such the enzymes of our approval procedures do resemble those already permitted by FSANZ both in function and in structure.

Figure 1 below shows an example of natural variation of alpha-amylases. The same holds for any other enzyme type. While significant differences in sequence amongst the various species exist, they all catalyse the same reaction and therefore fit under the same IUBMB entry. There will also be natural variation within one species. All this also applies to the enzymes under the current approval procedures by FSANZ:

% amino acid sequence identity	*B. amyloliquefaciens*	*B. licheniformis*	*G. stearothermophilus*	*A. niger*	*A. oryzae*	*Z. mays*	*O. sativa*	*H. vulgare*	*P. vulgaris*	*H. sapiens*
Bacillus amyloliquefaciens	100									
Bacillus licheniformis	80	100								
Geobacillus stearothermophilus	65	65	100							
Aspergillus niger	21	21	22	100						
Aspergillus oryzae	23	24	24	66	100					
Zea mays (corn)	24	26	25	28	27	100				
Oryza sativa (rice)	25	27	25	27	26	89	100			
Hordeum vulgare (barley)	25	23	24	25	28	70	69	100		
Phaseolus vulgaris (bean)	26	27	25	24	27	67	65	64	100	
Homo sapiens (human)	25	33	29	22	28	23	22	23	24	100

α-amylases in nature have divergent amino acid sequences but have the same catalytic activity and IUBMB number

Figure 1. Variation of enzymes in nature.

The expressed mature enzyme amino acid sequence of *Glycine max* beta-amylase (also known as 4-α-D-glucan maltohydrolase) shows a clear conserved PLN02801 sequence domain characteristic for beta-amylase activities, belonging to the Glycosyl hydrolase family 14.

A selection of beta-amylase sequences of the species listed on Schedule 18 of the ANZ Food Standards Code were retrieved from the UniProtKB database and analysed for homology. The highest homology between the beta-amylase enzyme subject of this dossier and analysed beta-amylase enzymes present on Schedule 18 of the ANZ Food Standards Code is 69% identity with the wheat beta-amylase. The identity between the FSANZ approved beta-

amylases from plant sources (malted cereals-wheat and barley, sweet potato) ranges from 59% (barley beta-amylase to sweet potato beta-amylase) to 81% (barley beta-amylase to wheat beta-amylase). Note that the UniProtKB database does not contain beta-amylase entries from *Bacillus subtilis* or *B. amyloliquefaciens*, but only contains *Bacillus cereus, Bacillus firmus, Bacillus circulans* beta-amylase entries, which are expected to show similar beta-amylase sequences to those of *B. subtilis* and *B. amyloliquefaciens*. Identity between *B. cereus, B. firmus, B circulans*, wheat, barley, and sweet potato beta-amylases range between 24% (*B. cereus* beta-amylase to wheat beta-amylase) to 82% (*B. firmus* beta-amylase to *B circulans* beta-amylase).

It is good to realise that the beta-amylase sequences within one species can show strain dependent amino acid sequence variability. Also, several species contain more than one beta-amylase encoding genes with different sequences (e.g. the model plant *Arabidopsis thaliana* contains nine beta-amylase sequences) (Thalmann et al, 2019).

3.2. Toxicity of the enzyme

β-amylase is naturally occurring in microorganisms and numerous other higher plants. Amylase preparations are mostly composed of different amylases, including beta-amylase. Amylases have a wide variety of applications in both food and pharmaceutical industries. They are used in the production of high-sugar syrups, baked goods (Agarwal, 2014), fruit juices and alcoholic beverages[1]. Other applications exist as washing and cleaning agents and in the production of textiles (Aiyer, 2005). These data demonstrate that humans are historically exposed to β-amylase.

As demonstrated earlier, β-amylase is produced from whey of non-GM soybean, a vegetable consumed by humans for centuries. Soybean has been used in Asia for centuries in traditional foods such as tofu, soymilk, tempeh and natto. Soybean and its products are widely used in foodstuff, not only as a food (e.g. tofu) but also as a technological aid (e.g. emulsifiers, texturizer). In recent years, breakthroughs in food science and processing have made it possible to use soybean ingredients for functional or nutritional purposes. This has greatly expanded the food processing industry's use of soybeans and soy-based ingredients.

Soybean (*Glycine max*) is extensively used all over the world due to its nutritional qualities. Numerous studies suggest that soy food consumption may contribute to lower rates of certain chronic diseases such as hormone dependent cancers, certain cardiovascular diseases, and osteoporosis.

However, *Glycine max* seeds are also known to contain different proteins displaying antinutritional and/or toxic effects, such as soybean agglutinin (an *N*-acetylgalactosamine-specific lectin), proteinase inhibitors (Kunitz- and Bowman–Birk-type trypsin and chymotrypsin inhibitors) and urease (Vasconcelos *et al*. 1997; 2001). Recently, two other toxic proteins were isolated from soybeans, soyatoxin and soybean toxin which are immunologically related to canatoxin, a toxic protein from *Canavalia ensiformis* (jackbean) seeds (Becker-Ritt *et al*. 2004).

Nevertheless, negative effects induced by these factors can be partially eliminated or inactivated with adequate heat-treatment (Vasconcelos *et al*. 2001).

[1] http://www.worthington-biochem.com/ba/default.html

3.3. Allergenicity of the enzyme

Bioinformatic analyses based on sequence homology determined that the SBA itself is unlikely to pose a risk of food allergenicity. Refer to Appendix B for additional information on the safety of the enzyme as to its allergenicity potential.

SBA preparation is produced from soybean, which is known to be a common food allergen. Consequently, it should be stated that the SBA preparation may contain traces of soy to prevent allergic reactions in consumers of food products treated with the enzyme preparation. SBA product literature discloses the use of soy protein in the production process and notes its allergen status. An allergen statement is given in Appendix A9.

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

As discussed in section 1.8, beta-amylase from soybean has not been reviewed by JECFA. Beta-amylase from soybean has been positive listed as permitted food additive for food manufacturing in Japan and China. EFSA has also published the safety evaluation of the food enzyme beta-amylase obtained from soybean (*Glycine max*), and concluded the food enzyme b-amylase obtained from soybean not to give rise to safety concerns under the intended conditions of use in starch processing.

Refer Appendix D for safety reports/approval letters.

4. Dietary exposure

Refer to Appendix C for further details

4.1. List of food or food groups likely to contain the enzyme or its metabolites

According to the food group classification system used in Standard 1.3.1-Food Additives Schedule 15 (15-5), SBA will be used in:

- 11.2. Sugars and sugar syrups

Products made with SBA, i.e. sugars and syrups, are also used as food ingredients, and would subsequently be used in production of all food categories where these ingredients are allowed.

4.2. Levels of residues in food

The proposed application rate of SBA in its intended application is listed below.

Application	Raw material	Recommended use levels (kg/MTDS)	Maximal recommended use levels (kg/MTDS)	Maximal recommended use levels (mg TOS/kg RM
Maltose production	Starch	0.15-1.15	1.15	919

The average TOS content in product is 79.9%.

DuPont N&B expects the SBA to be removed during the subsequent production and refining processes for its intended application. In starch processing, SBA performs its technological function during the saccharification step to facilitate the maltose formation. SBA is denatured by heat during a dedicated inactivation step or removed during subsequent carbon or ion exchange treatments.

Experimental data have shown significant removal (> 99%) of protein in the course of maltose syrup production. As presence of residual amounts of TOS after starch processing is negligible, dietary analysis is excluded (EFSA, 2017).

4.3. Likely level of consumption of foods or food groups not currently listed in the most recent Australian or New Zealand National Nutrition Surveys (NNSs)

Not applicable. SBA is not expected to be used in production of any foods or food groups that are currently not listed in NNSs. If such usage arises, an application would be made to inform FSANZ.

4.4. 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

The enzyme would be used as a processing aid in about:

- 1 % of sugar and sugar syrups

It is expected that SBA will be used in less than 1% of sugar and sugar syrups locally manufactured in Australia/New Zealand. Syrup imported to Australia/New Zealand could be also made with SBA by syrup producers overseas.

4.5. Levels of residues in food in other countries

Applications and levels of use of the SBA preparation in other countries is the same as presented in section 4.2. DuPont do note that the same enzyme could be commercialised by Nagase for manufacturing rice cake in Japan, as described by in EFSA assessment report (EFSA, 2017).

4.6. Likely current food consumption for foods where consumption has changed in recent years

Not applicable. Consumption of foods (including alcoholic drinks, non-alcoholic drinks) produced with SBA is not expected to have a significant change.

5. References

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