Annex 5

FORM FOR THE SUBMISSION OF SUBSTANCES TO BE EVALUATED BY JECFA

In completing this form, only brief information is required. The form may be retyped if more space is needed under any one heading provided that the general format is maintained.

Name of Substance(s):	**Transglucosidase/alpha-glucosidase from *Trichoderma reesei* expressing an Alpha-glucosidase Gene from *Aspergillus niger***
Question(s) to be answered by JECFA *(Provide a brief justification of the request in case of re-evaluations)*	Safety evaluation when used as processing aid.

1. Proposal for inclusion submitted by:

The Danish Veterinary and Food Administration
Head Office
Att: Jytte Kjaergaard
Stationsparken 31-33
DK 2600 Glostrup
Tel. +45 72 27 69 00

2. Name of substance; trade name(s); chemical name(s):

Name of the Substance	**classification 1**	**classification 2**
	Transglucosidase	**Alpha-glucosidase**
Enzyme name	1,4-alpha-glucan 6-alpha-glucosyltransferase	Alpha glucosidase
Other name	Oligoglucan-branching glucosyltransferse	Acid maltase, glucoinvertase, etc
Systematic name	(1→4)-alpha-D-Glucan:(1→4)-alpha-D-glucan(D-glucose) 6-alpha-D-glucosyltransferase	Alpha-D-Glucoside glucohydrolase
IUBMB No	2.4.1.24	3.2.1.20
CAS No	9030-12-0	9001-42-7
Reaction	Hydrolysis and transfer an alpha-D-glucosyl units of oligosaccharides and convert 1,g glucosidic linkage to t1,6 glucosidic linkages	Hydrolysis of terminal, non-reducing (1->4)-linked alpha-D-glucose residues with release of alpha-D-glucose
Trade Names	TRANSGLUCOSIDASE L-2000, FERMENZYME TL (main commercial names)	

3. Names and addresses of basic producers:

Danisco US Inc. (operating as DuPont Industrial Biosciences)
925 Page Mill Road
Palo Alto, CA 94304
UNITED STATES
Tel.: +1 650 846 7500

4. Has the manufacturer made a commitment to provide data?

Danisco US Inc. (operating as DuPont Industrial Biosciences) commits to provide data to support the proposal for inclusion of transglucosidase and α-glucosidase in the list of substances to be evaluated by JECFA.

5. Identification of the manufacturer that will be providing data (Please indicate contact person):

Danisco US Inc. (operating as DuPont Industrial Biosciences)
925 Page Mill Road
Palo Alto, CA 94304
UNITED STATES
Tel.: +1 650 846 7500

Attn.: Vincent J. Sewalt, PhD, Senior Director, Product Stewardship & Regulatory
vincent.sewalt@dupont.com

+1 650 846 5861

6. Justification for use:

The food enzyme catalyzes both hydrolytic and transfer reactions on incubation with α-D-gluco-oligosaccharides.

Transfer occurs most frequently to HO-6, producing isomaltose from D-glucose, and panose from maltose. The transfer can also occur to the HO-2 or HO-3 of D-glucose to form kojibiose or nigerose, or back to HO-4 to form maltose. The action on maltose produces equimolar concentration of panose and glucose.

As the result of α-glucosidase-transglucosidase reactions, the malto-oligosaccharides are converted to isomalto-oligosaccharides containing high proportions of glucosyl residues linked by an α-D-1,6 linkage from the non-reducing end.

In addition, the enzyme also hydrolyzes the terminal, non-reducing (1,4)-linked alpha-D-glucose residues with release of alpha-D-glucose.

In molasses, non-fermentable sugars including raffinose and stachyose are converted to sucrose, galactose, glucose and fructose, which can then be fermented into alcohol.

Industrial specific benefit:

For Isomalto-oligosaccharides (IMO) syrup production

- Convert the malto-oligosaccharides in starch to isomalto-oligosaccharides
- The only method to produce IMOs from starch to general knowledge

For potable alcohol, lysine, lactic acid and monosodium glutamate (MSG) processing:

- Convert the malto-oligosaccharides in starch from grains to isomalto-oligosaccharides
- Convert non-fermentable sugars including raffinose and stachyose into sucrose, galactose, glucose and fructose to be fermented

- Increase productivity
- Potential for higher alcohol yield
- Potential for use of less raw material

The effect of the enzymatic conversion is not noticeable in the final food.

7. Food products and food categories within the GSFA in which the substance is used as a food additive or as an ingredient, including use level(s):

Transglucosidase/α-glucosidase enzyme preparation is intended for use in the production of isomalto-oligosaccharides and in the manufacture of potable alcohol, lysine, lactic acid and MSG.

To obtain the desired effects of this enzyme, the recommended dose is as follows in accordance with current Good Manufacturing Practices (cGMPs):

- IMO production: 0.5-1.5 kg enzyme preparation/MT Dry Starch
- Potable alcohol process: 6-20 g enzyme preparation/MT Dry starch
- Lysine, lactic acid and MSG: 4 kg enzyme preparation/MT Dry starch

8. Is the substance currently used in food that is legally traded in more than one country? (Please identify the countries); or, has the substance been approved for use in food in one or more country? (Please identify the country(ies))

The enzyme preparation containing transglucosidase/α-glucosidase produced with this production organism is legally traded in the following countries:

- USA: GRAS Notice GRN 315 http://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices&id=315&sort=GRN_No&order=DESC&startrow=1&type=basic&search=315
- EU (except France, Denmark) based on due diligence and status as processing aid.
- South Korea (based on formal approval)

9. List of data available (please check, if available)

The production organism is from a safe strain as described in the decision tree in Pariza and Johnson, 2001[1]. However, to accommodate various registration requirements in different countries, a full toxicity program for food enzymes has been performed

Toxicological data

(i) Metabolic and pharmacokinetic studies

Not applicable.

(ii) Short-term toxicity, long-term toxicity/carcinogenicity, reproductive toxicity, and developmental toxicity studies in animals and genotoxicity studies

The following studies have been conducted in accordance with internationally accepted guidelines (OECD/EU/FDA) and do not give any concerns:

- Acute oral toxicity in rats
- Bacterial reverse mutation – Ames assay
- In *vitro* mammalian chromosomal aberration test performed with human lymphocytes
- 13 – week oral (gavage) toxicity study in rats

The conclusion of the safety studies can be summarized as follows:

[1] Pariza MW, Johnson EA; Evaluating the safety of microbial enzyme preparations used in food processing: update for a new century; Regul Toxicol Pharmacol 2001 Apr;33 (2):173-86.

The safety of t transglucosidase/α-glucosidase is assessed in a battery of toxicology studies investigating its acute oral, genotoxic and systemic toxicity potential.

Transglucosidase/α-glucosidase is not hazardous based on acute oral study according to the classification scenario in the Directive of the Commission 93/21/EEC of April 27, 1993 and the Globally Harmonized System of Classification and Labeling of Chemicals (GHS), 2007. In genotoxicity studies transglucosidase/α-glucosidase is not mutagenic, clastogenic or aneugenic.

Daily administration of α transglucosidase/α-glucosidase by oral gavage for 18 consecutive weeks did not result in adverse systemic toxicity or adverse effects on clinical chemistry, hematology, functional observation tests and macroscopic and histopathologic examinations. Under the conditions of this assay, the NOAEL (no observed adverse effect level) is established at the highest dose tested, 63.64 mg total protein/kg bw/day corresponding to 74.8 mg TOS/kg bw/day or 3230 MTGU/kg bw/day.

(iii) Epidemiological and/or clinical studies and special considerations

Not applicable.

(iv) Other data

None.

Technological data

(i) Specifications for the identity and purity of the listed substances (specifications applied during development and toxicological studies; proposed specifications for commerce)

The product conforms to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing as prepared by the Joint FAO/WHO Expert Committee on Food Additives at its sixty-seventh meeting for publication in FAO JECFA Monographs 3 (2006) and to the acceptance criteria, impurity limits, other test and other requirements for enzyme preparations listed in the Food Chemicals Codex, 9th edition[2]

(ii) Technological and nutritional considerations relating to the manufacture and use of the listed substance

The transglucosidase/α-glucosidase enzyme preparation from *Trichoderma reesei* will be used as a processing aid in the production of iso-malto-oligosaccharides and in the manufacture of potable alcohol, lysine, lactic acid and MSG. It hydrolyses and transfers α-D-gluco-oligosaccarides resulting isomalto-oligosaccharides from malto-oligosaccharides and fermentable sugars from molasses. The enzyme will be deactivated or denatured by the several following steps in the processing such as distillation and carbon treatment under certain condition (pH and temperature). No enzyme will be present in the final product.

Transglucosidase/α-glucosidase is a protein and any residual amounts remaining in food consumed would have the same nutritional value accordingly. However, the use levels of transglucosidase/α-glucosidase are very low. As with other enzymes that are currently approved and used as processing aids, use of this product would have an insignificant impact on the nutritional value of the food.

Intake assessment data

(i) Levels of the listed substance used in food or expected to be used in food based on technological function and the range of foods in which they are used

Transglucosidase/α-glucosidase is intended for use as processing aid in the IMO production and in the manufacture of potable alcohol, lysine, lactic acid and MSG.

[2] US Pharmacopeial. 2014 Enzyme preparations. Food Chemical Codex Edition 9, pp. 375-380. The United States Pharmacopeial Convention, Washington, DC.

IMO processing = 0.5 to 1.5 kg enzyme preparation/MT starch

Potable alcohol processing = 6 to 20 gg enzyme preparation/MT starch

Lysine processing = 4 kg enzyme preparation/MT starch

Lactic acid processing = 4 kg enzyme preparation/MT starch

MSG processing = 4 kg enzyme preparation/MT starch

(ii) Estimation of dietary intakes based on food consumption data for foods in which the substance may be used.

Under the worst case scenario, the cumulative daily exposure to transglucosidase/α-glucosidase from intake of syrup, potable alcohol, lactic acid, MSG and lysine is:

Syrup =	0.031 mg TOS/kg bw/day
Potable alcohol =	0.002 mg TOS/kg bw/day
Lactic acid =	0.008 mg TOS/kg bw/day
MSG =	0.059 mg TOS/kg bw/day
Lysine =	0.047 mg TOS/kg bw/day

Human Cumulative Exposure = 0.147 mg TOS/kg bw/day

Other information (as necessary/identified)

None

10. Date on which data could be submitted to JECFA

As soon as necessary.