Annex 2

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):	**Alpha-amylase from *Bacillus licheniformis* expressing a modified alpha-amylase gene from *Geobacillus stearothermophilus***
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 substance : Alpha-amylase from *Bacillus licheniformis* expressing a modified Alpha-amylase Gene from *Geobacillus stearothermophilus*

Trade names : SPEZYME ALPHA, SPEZYME CASSAVA (main commercial names)

Chemical names : IUBMB 3.2.1.1 and CAS number 9000-90-2

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?

DuPont Industrial Biosciences (Danisco US Inc.) commits to provide data to support the proposal for inclusion of the alpha-amylase 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.: Lisa Jensen, Regulatory Affairs Consultant

lisa.jensen@dupont.com +45 89435564

Alternative/Copy: mirjam.rademaker@dupont.com

6. Justification for use:

The food enzyme catalyses the endohydrolysis of (1→4)-alpha-D-glucosidic linkages in polysaccharides containing three or more (1→4)-alpha-linked D-glucose units with the main reaction products being maltodextrins, maltooligosacharides and glucose.

GC 358 (used as the general code for this enzyme product) contains a thermostable starch hydrolyzing alpha-amylase. It quickly reduces the viscosity of gelatinized starch, producing soluble dextrins and oligosaccharides under a variety of process conditions.
For grain processors, it offers the following benefits:
• Quick viscosity reduction allowing for higher solids
• Liquefaction pH's as low as 5.2
• Process flexibility
• Improved performance at low slurry temperatures

Industry specific benefits:
For starch processors: GC 358 rapidly lowers viscosity of gelatinized starch and allows therefore processing at high solid levels. This offers significant energy savings in the concentration of the final products by evaporation.
For ethanol producers: GC 358 reduces the viscosity of grain mashes rapidly and allows processing of high mash solids.
For brewing operators: GC 358 reduces the viscosity of the unmalted cereals rapidly allowing for high mash solids.

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):

GC 358 is intended for carbohydrate processing for the manufacture of HFCS (High Fructose Corn Syrups), cassava starch processing, wheat starch processing, cane sugar processing, and brewing and potable alcohol manufacture.

To obtain the desired effects of this enzyme, the recommended dose is 0.2 - 0.6 kg enzyme preparation/MT starch in accordance with current Good Manufacturing Practices (cGMPs).

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 Alpha-amylase produced with this production organism is approved in the following countries:

- France: The enzyme has been approved for its safety in France. It has passed already the European TRIS procedure, as can be seen here http://ec.europa.eu/growth/tools-databases/tris/en/search/?trisaction=search.detail&year=2015&num=308
  (End of Standstill: 17/09/2015). (See attachment)
  The publication in the French Official Journal is expected on short run.

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 world-wide, a full toxicity program for food enzymes has been performed according to the SCF guidelines for the evaluation of food enzymes[2].

[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.
[2] Opinion expressed by the Scientific Committee for Food on 11 April 1991, http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_27.pdf .

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 – Fixed dose procedure
- Sub-chronic 13 week toxicity in the rat
- Bacterial reverse mutation assay (Ames assay)
- In vitro mammalian cell gene mutation test(L5178/TK+/- Mouse Lymphoma Assay)
- In vitro mammalian cell micronucleus assay in human peripheral blood lymphocytes

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

The safety of GC 358 is assessed in a battery of toxicology studies investigating its acute oral, genotoxic and systemic toxicity potential. GC 358 is not acutely toxic by ingestion. Daily administration of GC 358 by gavage for 90 continuous days did not result in overt signs of systemic toxicity. A battery of genotoxicity assays was conducted and under the conditions of these assays GC 358 is not a mutagen or a clastogen.

(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.

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

The alpha-amylase enzyme preparation from *Bacillus licheniformis* GC 358 will be used as a processing aid in starch processing and in the manufacture of beer and potable alcohol. It quickly reduces the viscosity of gelatinized starch, producing soluble dextrins and oligosaccharides under a variety of process conditions. The action of the enzyme takes place at the beginning of the process to hydrolyse the starch for further processing. This step is followed by several steps of liquefaction and saccharification. The boiling process will inactivate the enzyme. No enzyme will be present in the end product due to distillation in the case of potable alcohol product.

Alpha-amylase is a protein and any residual amounts remaining in food consumed would have the same nutritional value accordingly. However, the use levels of Alpha-amylase 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

GC 358 is intended for carbohydrate processing for the manufacture of HFCS, cassava starch processing, wheat starch processing, cane sugar processing, and brewing and potable alcohol manufacture.

The proposed application rates of GC 358 are:

Cassava starch processing = 0.2 to 0.3 kg enzyme preparation/MT starch

Wheat starch processing = 0.4 to 0.6 kg enzyme preparation/MT starch

Brewing and alcohol processing = 0.2 to 0.45 kg enzyme preparation/MT starch

Carbohydrate processing = 0.2 to 0.45 kg enzyme preparation/MT starch

Cane sugar processing = 5 ppm active enzyme/MT starch

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

Based on the conservative calculation by means of the Budget method, based on an application of GC 358 containing 9.35% TOS at the maximum rate of 0.6 kg GC 358/MT starch, the maximum human daily intake of TOS from processed liquid foods (non-milk) and solid foods containing GC 358 is 0.315 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.

Attachment: *"Attachment_Application GC 358 JECFA_Avis de l'Anses_25FEB15"*