CODEX ALIMENTARIUS COMMISSION E

Food and Agriculture Organization of the United Nations

World Health Organization

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Agenda Item 8a **CX/FA 14/46/19**
February 2014

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON FOOD ADDITIVES

Forty-Sixth Session

Hong Kong, China, 17-21 March 2014

PROPOSALS FOR ADDITIONS AND CHANGES TO THE PRIORITY LIST OF FOOD ADDITIVES PROPOSED FOR EVALUATION BY JECFA (REPLIES TO CL 2013/12-FA)

Comments of Costa Rica, Egypt, European Union, Japan, Malaysia, Paraguay

COSTA RICA

Costa Rica welcomes the development of the document and has no comments.

EGYPT

EOS Comments

1. Some dye may be added to some foods as adulterated material as in spices which may also has harmful effects. So, as spices already used in most of the Egyptian diets.

We need to know the Egyptian diets.

We need to know the toxic effect of this dye (Sudan dye).

However, Egypt would like also to know a reference method to detect Sudan dye in food products and their toxic effect to protect the human health as in codex objections.

2. Some of food additives i.e (silver color E174, Golden color E175, Iron oxide red INS 172 (ii)) are used in food processing but we have no reference method used for detection and determination of the following dyes:

Priority list of substances proposed for Evaluation by JECFA

	Question (s) to be answered	Data availability (when/what)	Proposed by
*** Sudan Dyes**	Establishment of test method	Immediately	Egypt
Silver color E174	Establishment of test method	June 2014	Egypt
Golden color	Establishment of test method	June 2014	Egypt
*** Iron Oxide red INS 172 (ii)** **Iron Oxide black INS 172 (i)** **Iron Oxide yellow INS 172 (iii)**	Establishment of test method	April 2014	Egypt

EUROPEAN UNION

The European Union and its Member States are proposing to add the following substances to the priority list of substances proposed for evaluation by JECFA:

1) Asparaginase from *Aspergillus niger* expressing a modified gene from *Aspergillus niger*

2) Phospholipase A2 from pig pancreas expressed in *Aspergillus niger*

3) Glucose oxidase from *Penicillium chrysogenum* expressed in *Aspergillus niger*

4) Xylanase from *Talaromyces emersonii* expressed in *Aspergillus niger*

5) Rosemary extract (INS 392)

Annex 1- Asparaginase from *Aspergillus niger* expressing a modified gene from *Aspergillus niger*

Name of Compound(s):	**Asparaginase from *Aspergillus niger* expressing a modified gene from *Aspergillus niger***
Question(s) to be answered by JECFA *(kindly 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:

Ministry of Health, Welfare and Sport Nutrition
Health Protection and Prevention Department Parnassusplein 5
2511 VX The Hague
P.O. box 20350
2500 EJ The Hague
The Netherlands
Tel: +31 703407132

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

Name of compound : Asparaginase from *Aspergillus niger* expressing a modified gene from *Aspergillus niger*

Trade names : PreventASe XR

Chemical names : L-asparagine amidohydrolase; asparaginase II; L-asparaginase; colaspase; elspar; leunase; crasnitin; α-asparaginase; EC.3.5.1.1

3. Names and addresses of basic producers:

DSM Food Specialties
15 Rue des Comtesses
PO Box 239
59472 Seclin Cédex
France
Tel: 33 320964545
Fax: 33 320964500

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

Yes.

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

Dr. Mariella Kuilman
Regulatory Affairs
DSM Food Specialties
PO Box 1
2600 MA Delft
The Netherlands
Tel: 31 15279 3579
Fax: 31 15279 3614
E-mail: Mariella.Kuilman@DSM.com

6. Justification for use:

Acrylamide, which is a undesired food contaminant, is formed upon heating (e.g. baking) of asparagine- and sugar containing raw materials. The asparaginase enzyme preparation is used as a processing aid during food production to convert asparagine to aspartic acid in order to reduce acrylamide formation.

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

The enzyme preparation is used as processing aid in cereal and potato based products in accordance with current Good Manufacturing Practice (cGMP). The dosage of the enzyme varies between 1 and 115 mg Total Organic Solids (TOS)/kg flour or potato, depending on the specific application and raw material.

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

The enzyme preparation containing asparaginase from *Aspergillus niger* is authorized in the following countries:

- USA : GRN 428
- Canada : under evaluation, approval to be published Q1 2014

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]. To accommodate various registration requirements in different countries world-wide, a full toxicity program for food enzymes has been performed according to the EFSA CEF guidelines for the evaluation of food enzymes[2].

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:

- Test for mutagenic activity (Ames Test)
- Chromosomal aberration assay in Cultured peripheral human lymphocytes
- 13 weeks oral toxicity study in rats

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

The enzyme from *Aspergillus niger* shows no mutagenic and clastogenic activity. 13 weeks oral administration of the enzyme to rats did not cause in dose related findings. Therefore, the highest dose administered, 1254 mg TOS/kg body weight/day, is considered as the NOAEL.

(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 compounds (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, 7th edition.

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

The enzyme preparation from Aspergillus niger will be used as processing aid in the manufacture of cereal and potato based baked products. The action of the enzyme present in the preparation takes place in the dough preparation process step or in dipping or spraying step between blanching and partial drying in potato processing. During the further processing of the dough or potato products, baking, extrusion and/or frying, the enzyme activity is lost. No residual enzyme activity remains in the final product after baking or frying. The

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. EFSA (2009). Guidance of EFSA prepared by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. The EFSA Journal, 1305, 1-26. http://www.efsa.europa.eu/en/efsajournal/doc/1305.pdf, last visited on 30 August 2013.

use of the enzyme preparation as processing aid has no negative influence on the nutritional properties of the final product. In contrast, the acrylamide content will be reduced significantly.

Intake assessment data

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

Based on the dose of 1 – 77 mg TOS/kg cereal flour, and the fact that on average 1 kg final product is produced from 0.71 kg flour, the amount of TOS in the final product will be 0.7 – 55 mg TOS/kg.

Based on the dose of 10 – 115 mg TOS/kg potato (flour), and the fact that on average 1 kg final product is produced from 0.8 kg flour, the amount of TOS in the final product will be 8 - 92 mg TOS/kg.

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

Based on the conservative calculation by means of the Budget method, and assuming that the daily intake of processed foods is 50% of the total solid food intake, i.e. 0.025 kg/kg bw/day, the daily intake will be 0.01 – 1.15 mg TOS/kg bw/day.

Other information as necessary

None

10. Date on which data could be submitted to JECFA

As soon as necessary.

Annex 2- Phospholipase A2 from pig pancreas expressed in *Aspergillus niger*

Name of Compound(s):	**Phospholipase A_2 from pig pancreas expressed in *Aspergillus niger***
Question(s) to be answered by JECFA *(kindly 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:

Ministry of Health, Welfare and Sport
Nutrition, Health Protection and Prevention Department
Parnassusplein 5
2511 VX The Hague
P.O. box 20350
2500 EJ The Hague
The Netherlands
Tel: +31 703407132

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

Name of compound : Phospholipase A_2 from pig pancreas expressed in *Aspergillus niger*

Trade names : Maxapal A_2, Cakezyme smart, Purifine SB2, Purifine SB3, Purifine SB4, Purifine RS1, Purifine CN1

Chemical names : Phospholipase A_2 (EC 3.1.1.4)

3. Names and addresses of basic producers:

DSM Food Specialties
15 Rue des Comtesses
PO Box 239
59472 Seclin Cédex
France
Tel: 33 320964545
Fax: 33 320964500

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

Yes.

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

Dr Mariella Kuilman
Regulatory Affairs Manager
DSM Food Specialties
PO Box 1
2600 MA Delft
The Netherlands
Tel: +31 (0) 15 2793592
Fax: +31 (0) 15 2793614
E-mail: Mariella.Kuilman@dsm.com

6. Justification for use:

The enzyme phospholipase A_2 hydrolyzes natural phospholipids present in foodstuffs resulting in the formation of lyso-phospholipids that have emulsifying properties. The creation of lyso-phospholipids with the help of this enzyme preparation may be of benefit in baking (e.g. bread, muffins, biscuits, cakes) to improve batter viscosity, fine crumb structure, softness & enhance the volume and in egg processing for superior emulsifying properties (e.g. useful in dressings, spreads, sauces). In addition, the enzyme preparation is used during degumming of vegetable oils, where phospholipids can be separated more effectively from the oil.

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

The enzyme preparation is used as processing aid in baking, egg processing and oil degumming in accordance with current Good Manufacturing Practice (cGMP). The dosage of the enzyme varies between 60 – 2410 mg TOS/kg raw material depending on the specific application.

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

The enzyme preparation containing phospholipase A2 derived from a genetically modified strain of *Aspergillus niger* is authorized in the following countries:

- Australia : Food Standard 1.3.3 on Processing Aids
- Canada : Food standard B.16.100 Table V
- USA : GRN 183
- France : Annex 1 C, (Arrete October 19, 2006), last updated 12 September 2013
- Brazil : Resolucao-RDC No. 26, de 26 de Maio de 2009
- Mexico : Diario official lunes 16 de Julio de 2012, Anexo VI Enzimas

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[3]. However, to accommodate various registration requirements in different countries world-wide, a full toxicity program for food enzymes has been performed according to the EFSA guidelines for the evaluation of food enzymes[4].

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

3. 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.
4. Guidance of EFSA prepared by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. The EFSA Journal, 1305, 1-26.
http://www.efsa.europa.eu/en/efsajournal/doc/1305.pdf

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

- Test for mutagenic activity (Ames Test)
- Chromosomal aberration test, in vivo
- Human lymphocyte cytogenetic assay (in vitro micronucleus test)
- 13 weeks oral toxicity in rats

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

The enzyme from genetically modified *Aspergillus niger shows* no mutagenic and clastogenic activity.

13 weeks oral administration of the enzyme to rats did not cause any dose related findings. Therefore, the highest dose administered, 10000 mg test substance/kg body weight/day which is 1350 mg TOS/kg body weight/day is considered as the NOAEL.

(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 compounds (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, 8th edition.

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

The enzyme preparation from genetically modified *Aspergillus niger* will be used as processing aid in the manufacturing of bakery, processed egg products and oil degumming. The enzyme functions in early stage of baking and is inactivated at temperature above 65 ^{0}C. During egg processing, the enzyme is not functional due to lack of substrate and low pH conditions. In degumming of vegetable oils, the enzyme will end up in water phase whereas the oil is the product phase that will end up in final food applications. Hence it's clear that in all applications, no enzyme activity remains in the final food. The use of the enzyme preparation as processing aid has no influence on the nutritional properties of the final product.

Intake assessment data

(i) Levels of the listed compound used in food or expected to be used in food based on technological function and the range of foods in which they are used. The dosage of the enzyme varies between 300 – 1205 mg TOS/kg flour, 1205 – 2410 mg TOS/kg egg yolk and 60 – 121 mg TOS/kg crude oil depending on the specific application.

(ii) Estimation of dietary intakes based on food consumption data for foods in which the compound may be used. The calculated dietary intake is based on maximum use levels in final food. Thus for baking it's 952 mg TOS/kg bread and for egg processing it's 118mg TOS/kg dressing.

In the case of oil degumming, it is assumed that nothing of TOS will end up in the final product since the enzyme will end up completely in the water phase whereas the oil phase is the consumed final product.

Other information as necessary

None

10. Date on which data could be submitted to JECFA

As soon as necessary.

Annex 3- Glucose oxidase from *Penicillium chrysogenum* expressed in *Aspergillus niger*

Name of Compound(s):	**Glucose oxidase from *Penicillium chrysogenum* expressed in *Aspergillus niger***
Question(s) to be answered by JECFA *(kindly 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:

Ministry of Health, Welfare and Sport
Nutrition, Health Protection and Prevention Department
Parnassusplein 5
2511 VX The Hague
P.O. box 20350
2500 EJ The Hague
The Netherlands
Tel: +31 703407132

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

Name of compound : glucose oxidase from *Penicillium chrysogenum* expressed in *Aspergillus niger*

Trade names : BakeZyme® Go Pure

Chemical names : glucose oxidase (EC 1.1.3.4)

3. Names and addresses of basic producers:

DSM Food Specialties
15 Rue des Comtesses
PO Box 239
59472 Seclin Cédex
France
Tel: 33 320964545
Fax: 33 320964500

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

Yes.

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

Dr Jack Reuvers
Regulatory Affairs
DSM Food Specialties
PO Box 1
2600 MA Delft
The Netherlands
Tel: 31 15279
Fax: 31 152793614
E-mail: J.Reuvers@dsm.com

6. Justification for use:

The enzyme preparation is used in baking. Glucose oxidase helps to form inter-protein bonds in the dough, which strengthen the dough and increase its gas-retaining capacity. As a result, the dough has better handling properties. Moreover, bread of consistent quality and improved crumb structure is obtained. In certain baked products (e.g. croissants), an increase of the volume can be obtained.

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

The enzyme preparation is used as processing aid in baking in accordance with current Good Manufacturing Practice (cGMP). The dosage of the enzyme varies between 0.08 and 0.3 mg Total Organic Solids (TOS)/kg flour, depending on the specific application.

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

The enzyme preparation containing glucose oxidase from genetically modified strain of *Aspergillus niger* is not yet authorized in any country however registration process is ongoing the following countries:

- EU : Dossier has been submitted and validated by the EU Commission
- Denmark : Dossier has been submitted
- Franc : Dossier has been submitted
- US : GRAS self-affirmation has been prepared

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[5]. To full fill various registration requirements in different countries world-wide, a full toxicity program for food enzymes has been performed according to the EFSA guidelines for the evaluation of food enzymes[6].

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):

- Test for mutagenic activity (Ames Test)
- *In vitro* Mammalian Chromosome Aberration Test
- *In Vivo* Mammalian Erythrocyte Micronucleus Test
- 13 weeks dietary toxicity study in rats

The studies are ongoing. All the studies will be completed by June 2014 and final reports of these studies will be available before December 2014.

However, we would like to point out that the toxicological studies have been performed for regulatory reasons only. The safety of glucose oxidase from the genetically modified strain of *Aspergillus niger* for human consumption is based on the following factors:

- The long history of safe use: Glucose oxidase from *Penicillium chrysogenum* is an enzyme present in nature and described in literature since 1942. Its characteristics and uses are described in many publications and textbooks.
- The safety of the producing organism: *Aspergillus niger* has been used for the production of many different enzymes of which a number do have the GRAS-status. The current producing organism is from the same strain lineage (Safe Strain Lineage Concept).
- The production process, which is done under current Good Manufacturing Practice (cGMP) and Hazard Analysis of Critical Control Points (HACCP).
- The intrinsic properties of enzymes: their composition of amino acids and their digestibility in human. Many food enzyme preparations have been evaluated and accepted by the FDA, JECFA, and European national authorities. In particular, glucose oxidase from the classical *A. niger* is an enzyme permitted in Canada, Australia, Mexico, Brazil, China, Japan, US and also listed in the CODEX list of JECFA approved enzymes.

5. 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.
6. Guidance of the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids (CEF) on the Submission of a Dossier on Food Enzymes for Safety Evaluation by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids, EFSA Scientific Opinion, The EFSA Journal (2009) 1305, 1-26

- Glucose oxidase from *Penicillium chrysogenum* has been toxicologically evaluated in repeated 90-day repeated oral toxicity study and in genotoxicity studies and has shown neither toxic nor genotoxic effects (Konishi T, et al, Reg. Tox. Pharmacol. 2013; 66: 13-23).

Based on the above considerations, and on the identical production process used for glucose oxidase from the classical A. niger , the enzyme is considered safe for use in humal oral consumption.

(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 compounds (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, 8th edition.

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

The enzyme preparation from the genetically modified strain of *Aspergillus niger* will be used as processing aid in baking. The function of the enzyme present in the preparation takes place during mixing, proofing and in the early stage of baking. During the baking process enzyme protein will be inactivated and denatured. So no residual glucose oxidase activity remains in the finished products. The use of the enzyme preparation as processing aid has no influence on the nutritional properties of the final product.

Intake assessment data

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

Based on the dose of 0.08-0.3 mg TOS/kg flour, and the fact that 1 kg flour results in 1.1 kg bread or other baked products, the amount of TOS in the final product will be 0.07-0.27 mg TOS/ kg bread or other baked products.

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

Based on the conservative calculation by means of the Budget method, and assuming that the daily intake of bread and other baked products is = 0.0125 kg/kg bw/day, the daily intake will be 0.9 – 3.4 µg TOS/kg bw/day.

Other information as necessary

None

10. Date on which data could be submitted to JECFA

As soon as necessary.

Annex 4- Xylanase from *Talaromyces emersonii* expressed in *Aspergillus niger*

Name of Compound(s):	**Xylanase from *Talaromyces emersonii* expressed in *Aspergillus niger***
Question(s) to be answered by JECFA *(kindly 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:

Ministry of Health, Welfare and Sport
Nutrition, Health Protection and Prevention Department
Parnassusplein 5
2511 VX The Hague
P.O. box 20350
2500 EJ The Hague
The Netherlands
Tel: +31 703407132

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

Name of compound : Xylanase from *Talaromyces emersonii* expressed in *Aspergillus niger*

Trade names : Filtrase BX (brewing application), Bakezyme FXP (baking application)

Chemical names : endo-1,4-β-xylanase (EC 3.2.1.8)

3. Names and addresses of basic producers:

DSM Food Specialties
15 Rue des Comtesses
PO Box 239
59472 Seclin Cédex
France
Tel: 33 320964545
Fax: 33 320964500

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

Yes.

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

Dr Jack Reuvers
Regulatory Affairs
DSM Food Specialties
PO Box 1
2600 MA Delft
The Netherlands
Tel: 31 15279
Fax: 31 152793614
E-mail: J.Reuvers@dsm.com

6. Justification for use:

The enzyme preparation is used in beer brewing and other fermented beverages as well as in baking to hydrolyze arabinoxylans in cereals (e.g. malt, barley, wheat).

Insufficiently hydrolysed of cereal cell wall components such as arabinoxylans are viscous and reduce the effectiveness of wort and beer filtration therefore use of the enzyme preparation will lead to faster and more predictable lautering or mash filtration, increased flexibility in the choice of raw materials, higher brewing yield, faster beer filtration and reduced consumption of beer filtration aids (e.g. silica gels).

The enzyme preparation is also used in the manufacturing of bakery products such as, but not limited to, bread, biscuits, steamed bread, cakes, pancakes, tortillas, wafers and waffles. Arabinoxylans provide functional properties during bread making due to their ability to interact with gluten, bind water and provide dough viscosity. Limited hydrolysis of the water-unextractable arabinoxylans with the help of the enzyme preparation results in solubilized arabinoxylans with lower molecular weights, which improves the functional

baking properties of these polysaccharides, facilitate the handling of the dough (improved extensibility and stability), improve the dough's structure and behaviour during the baking step, ensure an uniform and increased volume and an improved crumb structure.

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

The enzyme preparation is used as processing aid in beer brewing and other fermented beverages as well as in baking in accordance with current Good Manufacturing Practice (cGMP).

The dosage of the enzyme varies between 1.4 and 5.6 mg Total Organic Solids (TOS)/ liter beer or other fermented beverages, depending on the specific application.

The dosage of the enzyme varies between 1.6 and 23.7 mg Total Organic Solids (TOS)/ kg bread or other baking product, depending on the specific application.

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

The enzyme preparation containing xylanase derived from the genetically modified strain of *Aspergillus niger* is currently not approved in countries with an enzyme legislation.

However the registration procedure will be started in 2014 in EU.

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[7]. To full fill various registration requirements in different countries world-wide, a full toxicity program for food enzymes has been performed according to the EFSA guidelines for the evaluation of food enzymes[8].

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:

- Test for mutagenic activity (Ames Test)
- *In vitro* Mammalian Chromosome Aberration Test 13 weeks oral toxicity study in rats
- 13 weeks oral toxicity study in rats

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

The enzyme from *Aspergillus niger* shows no mutagenic and clastogenic activity.

The 13 weeks oral administration of the enzyme to rats did not cause dose related findings. Therefore, the highest dose administered, 1850 mg TOS/kg body weight/day, is considered as the NOAEL.

(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 compounds (specifications applied during development and toxicological studies; proposed specifications for commerce).

7. 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.
8. Guidance of the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids (CEF) on the Submission of a Dossier on Food Enzymes for Safety Evaluation by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids, EFSA Scientific Opinion, The EFSA Journal (2009) 1305, 1-26

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, 8th edition.

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

The enzyme preparation from *Aspergillus niger* will be used as processing aid in the manufacture of beer and other fermented beverages as well as in baking. In brewing, the function of the enzyme present in the preparation takes place in the malting process step during the early stage of the brewing process. During the wort boiling step in the beer production process, the enzyme activity is lost. In baking, the function of the enzyme present in the preparation takes place during mixing, proofing and in the early stage of the baking process. During the baking process the enzyme protein will be inactivated and denatured. So no residual xylanase activity remains in the finished products.

The use of the enzyme preparation as processing aid has no influence on the nutritional properties of the final product.

Intake assessment data

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

The amount of TOS per 1 liter of beer or other fermented beverages will be 1.4-5.6 mg TOS/ l beer.

The amount of TOS per 1 kg baking product will be 1.6-23.7 mg TOS/ kg baking product.

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

Based on the conservative calculation by means of the Budget method, and assuming that the daily intake of beer and/or fermented beverage is comparable with the amount of soft drinks, i.e. 0.025 L/kg bw/day, the daily intake will be 0.035 – 0.14 mg TOS/kg bw/day.

Based on the conservative calculation by means of the Budget method, and assuming that at least 50% of foods containing the enzyme are processed, i.e. 0.0125 kg/kg bw/day, the daily intake will be 0.02-0.3 mg TOS/kg bw/day.

Other information as necessary

None

10. Date on which data could be submitted to JECFA

As soon as necessary.

Annex 5- Rosemary extract (INS 392)

Name of Compound(s):	**INS 392 Rosemary extract**
Question(s) to be answered by JECFA *(kindly provide a brief justification of the request in case of re-evaluations)*	Safety assessment when used as a food additive (antioxidant)

1. Proposal for inclusion submitted by:

Naturex SA
Site D'Agroparc
BP 1218
84911 Avignon
Cedex 9
France

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

Extract of rosemary leaf, Rosemary extract (*Rosmarinus officinalis*)

3. Names and addresses of basic producers:

Naturex SA
Site D'Agroparc
BP 1218
84911 Avignon
Cedex 9
France

The producer is represented by:

Nigel Baldwin BSc, MIFST, CSci
Director, Scientific and Regulatory Consulting, Europe
Intertek Cantox
Chemicals and Pharmaceuticals
Mob: +44 7836 293 834
Tel: +44 1252 39 24 68
Email: nigel.baldwin@intertek.com
Website: www.intertek.com/food/consulting
Skype: nigel.baldwin.intertek

Room 1036, Building A8
Cody Technology Park
Ively Road
Farnborough
Hampshire
GU14 0LX
UK

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

Yes.

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

On behalf of the manufacturer (Naturex SA – see above) all data will be provided by:

Nigel Baldwin BSc, MIFST, CSci
Director, Scientific and Regulatory Consulting, Europe
Intertek Cantox
Chemicals and Pharmaceuticals
Mob: +44 7836 293 834
Tel: +44 1252 39 24 68
Email: nigel.baldwin@intertek.com
Website: www.intertek.com/food/consulting
Skype: nigel.baldwin.intertek

Room 1036, Building A8
Cody Technology Park
Ively Road
Farnborough
Hampshire
GU14 0LX
UK

6. Justification for use:

Antioxidants are food additives, which prolongs the shelf-life of foods by protecting against deterioration caused by oxidation. Rosemary extract is derived from Rosmarinus officinalis L. and contain several compounds which have been proven to exert antioxidative functions. These compounds belong mainly to the classes of phenolic acids, flavonoids, diterpenoids and triterpenes. The principal antioxidative components of the extracts are the phenolic diterpenes carnosol and carnosic acid.

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

The relevant GSFA food categories for INS 392 Rosemary extract are listed below in the table 7.1

Table 7.1 GSFA food categories relevant for the use of INS 392 Rosemary extract			
Food Category[a]	**Sub-category**	**Use level (mg/kg)**[b]	**Notes**
1.0 Dairy products and analogues	1.5.1 Milk powder and cream powder (plain)	30	Dried milk for the manufacturing of ice cream
		200*	Milk powder for vending machines
2.0 Fats and oils and fat emulsions	2.1.3 Lard, tallow, fish oil and other animal fats	50*	Fats and oils for the professional manufacture of heat-treated foodstuffs; Frying oil and frying fat, excluding olive oil and olive pomace oil; fish and algal oils; Lard beef, poultry, sheep and porcine fat
	2.1.2 Vegetable oils and fats	30*	Vegetable oils (excluding virgin oils and olive oils) and fat where the content of PUFA is higher than 15% w/w of the total fatty acid, for the use in non heat treated food products
	2.2.2 Fat spreads, dairy fat spreads and blended spreads	30*	
	2.1.2 Vegetable oils and fats	50*	Only fats and oils for the professional manufacture of heat treated foods
4.0 Fruit and vegetables, seaweed and nuts and seeds	4.2.2.6 Vegetable, seaweed and nut and seed pulps and preparations other than food category 4.2.2.5	200	Only seaweed based fish roe analogues
	4.2.2.5 Vegetable, seaweed and nut and seed purees and spreads (e.g. peanut butter)	200*	
	4.2.2.2 Dried vegetables, seaweeds and nuts and seeds	200	Dehydrated potato products
5.0 Confectionery	5.3 Chewing gum	200	
	5.4 Decorations, toppings (non-fruit) and sweet sauces	100*	Sauces only
7.0 Bakery wares	7.2 Fine bakery wares and mixes	200*	
8.0 Meat and meat products, including poultry and game	8.2.1 Non heat treated processed meat, poultry and game products in whole pieces or cuts	15*	Only meat with a fat content not higher than 10%, excluding dried sausages
		150*	Only meat with a fat content higher than 10%, excluding dried sausages
	8.3.1 Non heat treated processed comminuted meat, poultry and game products	100	Only dried sausages
		150	Only dehydrated meat

Table 7.1 GSFA food categories relevant for the use of INS 392 Rosemary extract			
Food Category[a]	**Sub-category**	**Use level (mg/kg)**[b]	**Notes**
	8.2.2 Heat treated processed meat, poultry and game products in whole pieces or cuts	15*	Only meat with a fat content not higher than 10%, excluding dried sausages
		150*	Only meat with a fat content higher than 10%, excluding dried sausages
	8.3.2 Heat treated processed comminuted meat, poultry and game products	100	Only dehydrated sausages
		150	Only dehydrated meat
9.0 Fish and fish products, including mollusks, crustaceans and echinoderms	9.2 Processed fish and fishery products, including mollusks, crustaceans and echinoderms	15	Only fish and fishery products, including mollusks and crustaceans with a fat content not higher than 10%
		150*	Only fish and fishery products, including mollusks and crustaceans with a fat content higher than 10%
10.0 Eggs and egg products	10.2 Egg products	200	
12.0 Salts, spices, soups, sauces, salads, protein products	12.2.2 Seasonings and condiments	200*	
	12.4 Mustard s	100*	
	12.5 Soups and broths	50	
	12.6 Sauces and like products	100*	
15.0 Ready-to-eat savouries	15.1 Snacks - potato, cereal, flour, or starch-based	50*	
	15.2 Processed nuts, including coated nuts and nut mixtures	200*	
13.0 Foodstuffs intended for particular nutritional uses	13.6 Food supplements	400	

*Use level expressed on fat basis
[a] Grouped in food uses categories according to the Codex GSFA food categorisation system for food additives http://www.codexalimentarius.net/gsfaonline/index.html
[b] Expressed as sum of carnosol and carnosic acid

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

INS 392 Rosemary extract is permitted in the EU as an antioxidant in several food categories.

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

We will be able to provide all of the original technical and toxicology data that resulted from our application to the EU which resulted in the EFSA Opinion on the Use of rosemary extracts as a food additive - Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food

http://www.efsa.europa.eu/en/efsajournal/pub/721.htm

Please refer to the reference list of this document.

JAPAN

Name of Substance(s):	**Aspartame (Aspartyl phenylalanine methyl ester: APM; INS No. 951)**
Question(s) to be answered by JECFA *(kindly provide a brief justification of the request in case of re-evaluations)*	Revision of specifications ((1)Change of test of 5-bezyl-3,6-dioxo-2-piperazine acetic acid, (2) Change of test of other optical isomers)

1. Proposal for inclusion submitted by:

Japan

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

Generic name: Aspartame

Chemical name: N-L-alpha-aspartyl-L-phenylalanine-1-methyl ester

3. Names and addresses of basic producers:

Name: Ajinomoto Co., Inc.
Address: 1-15-1, Kyobashi, Chuo-ku, Tokyo, 104-8315, Japan

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

Yes

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

The producer shown in item 3 will provide the data.
[Contact person] Name: Yoko OBAYASHI
Department: Quality Assurance & External Scientific Affairs Dept.
TEL:+81-3-5250-8184 FAX: +81-3-5250-8403
e-mail address: youko_oobayashi@ajinomoto.com

6. Justification for use:

sweetener

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):

Typical food categories and use level of aspartame as sweetener

Category Number	Food Category	Max level
01.1.2	Dairy-based drinks, flavoured and/or fermented (e.g., chocolate milk, cocoa, eggnog, drinking yoghurt, whey-based drinks)	600 mg/kg
01.7	Dairy-based desserts (e.g., pudding, fruit or flavoured yoghurt)	1000 mg/kg
13.3	Dietetic foods intended for special medical purposes (excluding products of food category 13.1)	1000 mg/kg
13.4	Dietetic formulae for slimming purposes and weight reduction	800 mg/kg
13.6	Food supplements	5500 mg/kg
14.1.4	Water-based flavoured drinks, including "sport," "energy," or "electrolyte" drinks and particulated drinks	600 mg/kg

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))

Yes. Aspartame is used all over the world including USA, Japan, Europe.

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

Toxicological data

(i) Metabolic and pharmacokinetic studies

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

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

(iv) Other data

Technological data

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

Revision of specifications ((1)Change of test of 5-bezyl-3,6-dioxo-2-piperazine acetic acid, (2) Change of test of other optical isomers)

The analytical method of “5-bezyl-3,6-dioxo-2-piperazine acetic acid (DKP)” and “other optical isomers” in the purity test of the JECFA specification of aspartame has not yet been changed since the JECFA specification was established in 1981.

DKP is analyzed using gas chromatograph in the present JECFA specifications. Since the peak of the other substance was detected closely to that of DKP with the consequence of interrupting the precise quantification of DKP, it is preferable to change the gas-chromatographic assay to the HPLC method which was already adopted by FCC.

The other optical isomers in the sample are analyzed using amino acid analyzer equipped with strong cation exchange column. Since this column is not commercially available anymore, the revision of the analytical method for “other optical isomers” in the specification is necessary. Since chiral column is world-wide used to separate optical isomers, we developed and validated simple HPLC system using chiral column for analyzing other optical isomers in the sample.

We would like to propose replacement of the analytical method of “5-bezyl-3,6-dioxo-2-piperazine acetic acid” and “other optical isomers” in the purity test of the JECFA specification by the developed HPLC methods.

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

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

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

Other information as necessary

10. Date on which data could be submitted to JECFA

Until December 1, 2014.

MALAYSIA

Name of Substance(s):	**Steviol Glycosides**
Question(s) to be answered by JECFA *(kindly provide a brief justification of the request in case of re-evaluations)*	Amend the existing JECFA specification(2010) to i. include rebaudioside M and rebaudioside E; ii. delete the requirement for stevioside and/or rebaudioside A as the primary steviol glycosides in stevia preparations

1. Proposal for inclusion submitted by:

Malaysia

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

Additional Steviol Glycosides to be included in the existing JECFA specification(2010) are

i. Rebaudioside M (Chemical name:13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid,2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester)

ii. Rebaudioside E (Chemical name:13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)-oxy]kaur -16-en-18-oic acid, 2-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester)

Appendix A outlines the proposed amendment of the JECFA specification (2010)

3. Names and addresses of basic producers:

PureCircle SdnBhd,
Techpark@ENSTEK
PT 23419, LengkukTeknologi
71760 Bandar ENSTEK
Negeri Sembilan, Malaysia

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

Yes

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

Sidd Purkayastha, Ph.D.
VP, Business Development & Regulatory Affairs
PureCircle Limited
915 Harger Road, Suite 250
Oak Brook, Illinois 60523
sidd.purkayastha@purecircle.com
+1 - 630-361-0374 (Office)
+1 - 630-480-4365 (Fax)

6. Justification for use:

An amendment to the JECFA specification is justified based on the availability of rebaudioside M and rebaudioside E in commercially available stevia extracts. In fact,Rebaudioside E is currently listed in the European Union's steviol glycoside specification.

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):

The existing provision of food products, food categories, and use levels previously adopted for steviol glycosides should apply as the proposed work is only to amend the specification for steviol glycosides. A few of these categories are highlighted below. For a complete listing of all food products, food categories and use levels, please see the GSFA for steviol glycosides.

Category	**Maximum Use Level (as steviol equivalents)**
Non-alcoholic, water-based flavored drinks	200 ppm
Confectionary, with no added sugar	700 ppm
Jams or jellies, energy reduced	360 ppm
Chewing gum	3,500 ppm

Sauces	350 ppm
Yogurt	330 ppm

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)

In the United States, Reb M is considered Generally Recognized as Safe (GRAS), US FDA (GRN 473).

In Europe, Reb E is approved as one of the 10 permitted glycosides that may comprise the assay value of "not less than 95% purity".

Both rebaudioside M and rebaudioside E can be found in commercially available stevia extracts sold in all major countries (US, EU countries, China, Japan, Mexico, Australia, etc) However, according to the current JECFA specification, neither of these steviol glycosides are in the list of 9 permitted glycosides that may comprise the assay value of "not less than 95% purity".

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

Toxicological data

The toxicology data to support this proposal are available and can be provided upon request. Briefly, the data supporting this amendment include a)new in vitro metabolism data for various steviol glycosides including Reb M (published in peer-reviewed journal); b)new subchronic toxicity data

Technological data

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

The current specification for steviol glycosides was prepared at the 73rd JECFA (2010) and published in FAO JECFAMonographs 10 (2010). This supersedes the specifications prepared atthe 69th JECFA (2008) and published in FAO JECFA Monographs 5 (2008).

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

The additive steviol glycosides are of principal interest for its sweetening properties. Steviol glycosides are known to have a solubility ranging between "freely to slightly soluble in water" rather than only "freely soluble in water" (reference available). With regards to its individual steviol glycosides, both Reb M and Reb E have sweetness properties, with reb M having a potency that is 350x sweeter than sucrose (data available). Both steviol glycosides are thermally and hydrolytically stable for use in a variety of foods, including acidic beverages, under normal conditions of processing and storage (data available).

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

The proposed changes are for the specifications only. There are no changes to the categories/use levels in the GSFA.

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

Since no changes in the categories or use levels for steviol glycosides are being requested, the dietary intake assessment in the 2008 review are still appropriate.

10. Date on which data could be submitted to JECFA.

June, 2014

Appendix- A

STEVIOL GLYCOSIDES

DEFINITION (Para 2)

~~Stevioside and rebaudioside A are the component glycosides of principal interest for their sweetening property. Associated glycosides include rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside and steviolbioside which are generally present in preparations of steviol glycosides at levels lower than stevioside or rebaudioside A.~~

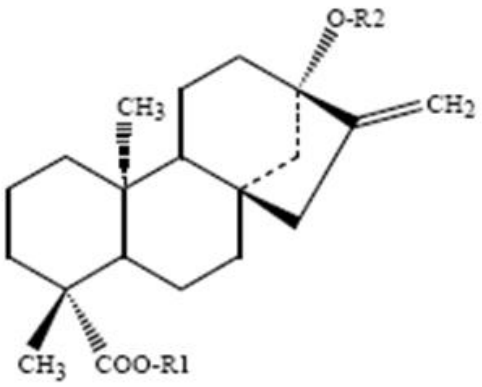
Stevioside, rebaudioside A, rebaudiosideB,rebaudiosideC,rebaudiosideD, rebaudioside E,rebaudiosideF, rebaudioside M, dulcosideA, Rubusoside and steviolbioside are generally present in preparations of steviol glycosides.

Chemical name/ C.A.S number/ Chemical formula

The chemical name and CAS number for each of the 11 steviol glycosides.

Common Name	**Chemical Name**	**CAS No.**
Rebaudioside A	13-[(2-O-β–D-glucopyranosyl-3-O-β–D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	58543-16-1
Stevioside	13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	57817-89-7
Rebaudioside B	13-[(2-O-β–D-glucopyranosyl-3-O-β–D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid	58543-17-2
Rebaudioside C	13-[(2-O-α–L-rhamnopyranosyl-3-O-β–D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	63550-99-2
Rebaudioside D	13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester	63279-13-0
Rebaudioside E	**13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur -16-en-18-oic acid, 2-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester**	**63279-14-1**
Rebaudioside F	13[(2-O-β-D-xylofurananosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	438045-89-7
Rebaudioside M	**13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester**	**1220616-44-3**
Rubusoside	13-β-D-glucopyranosyloxykaur-16-en-18-oic acid, β-D-glucopyranosyl ester	64849-39-4
Steviolbioside	13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid	41093-60-1
Dulcoside A	13-[(2-O-α–L-rhamnopyranosyl-β–D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	64432-06-0

Structural Formula The eleven named steviol glycosides:



Molecular Weight, Chemical Formula, and R-Groups in Backbone Structure of Steviol Glycosides				
Steviol Glycoside	**Molecular Weight**	**Chemical Formula**	**R-Groups in Backbone Structure**	
			R_1	**R_2**
Stevioside	804.88	$C_{38}H_{60}O_{18}$	β-Glc	β-Glc-β-Glc(2→1)
Rebaudioside A	967.01	$C_{44}H_{70}O_{23}$	β-Glc	β-Glc-β-Glc(2→1) | β-Glc(3→1)
Rebaudioside B	804.88	$C_{38}H_{60}O_{18}$	H	β-Glc-β-Glc(2→1) | β-Glc(3→1)
Rebaudioside C	951.02	$C_{44}H_{70}O_{22}$	β-Glc	β-Glc-α-Rha(2→1) | β-Glc(3→1)
Rebaudioside D	1,129.15	$C_{50}H_{80}O_{28}$	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1) | β-Glc(3→1)
Rebaudioside E	**967.01**	**$C_{44}H_{70}O_{23}$**	**β-Glc-β-Glc(2→1)**	**β-Glc-β-Glc(2→1)**
Rebaudioside F	936.99	$C_{43}H_{68}O_{22}$	β-Glc	β-Glc-β-Xly(2→1) | β-Glc(3→1)
Rebaudioside M	**1,291.3**	**$C_{56}H_{90}O_{33}$**	**β-Glc-β-Glc(2→1)** **|** **β-Glc(3→1)**	**β-Glc-β-Glc(2→1)** **|** **β-Glc(3→1)**
Dulcoside A	788.88	$C_{38}H_{60}O_{17}$	β-Glc	β-Glc-α-Rha(2→1)
Rubusoside	642.73	$C_{32}H_{50}O_{13}$	β-Glc	β-Glc
Steviolbioside	642.73	$C_{32}H_{50}O_{13}$	H	β-Glc-β-Glc(2→1)

Steviol (R1=R2=H) is the aglycone of the steviolglycosides.Glc,Rha and Xyl represent, respectively, glucose, rhamnose and xylose sugar moieties.

METHOD OF ASSAY

~~fX is the ratio of the formula weight of X to the formula weight of stevioside: 1.00 (stevioside), 1.20 (rebaudioside A), 1.00 (rebaudioside B), 1.18 (rebaudioside C), 1.40 (rebaudioside D), 1.16 (rebaudioside F), 0.98 (dulcoside A), 0.80 (rubusoside) and 0.80 (steviolbioside).~~

~~Calculate the percentage of total steviol glycosides (sum the nine percentages).~~

fX is the ratio of the formula weight of X to the formula weight of stevioside: 1.00 (stevioside), 1.20 (rebaudioside A), 1.00 (rebaudioside B), 1.18 (rebaudioside C), 1.40 (rebaudioside D), **1.20 (rebaudioside E),** 1.16 (rebaudioside F), **1.60 (rebaudioside M)**,0.98 (dulcoside A), 0.80 (rubusoside) and 0.80 (steviolbioside).

Calculate the percentage of total steviol glycosides (sum the eleven percentages).

Replace with new figure:

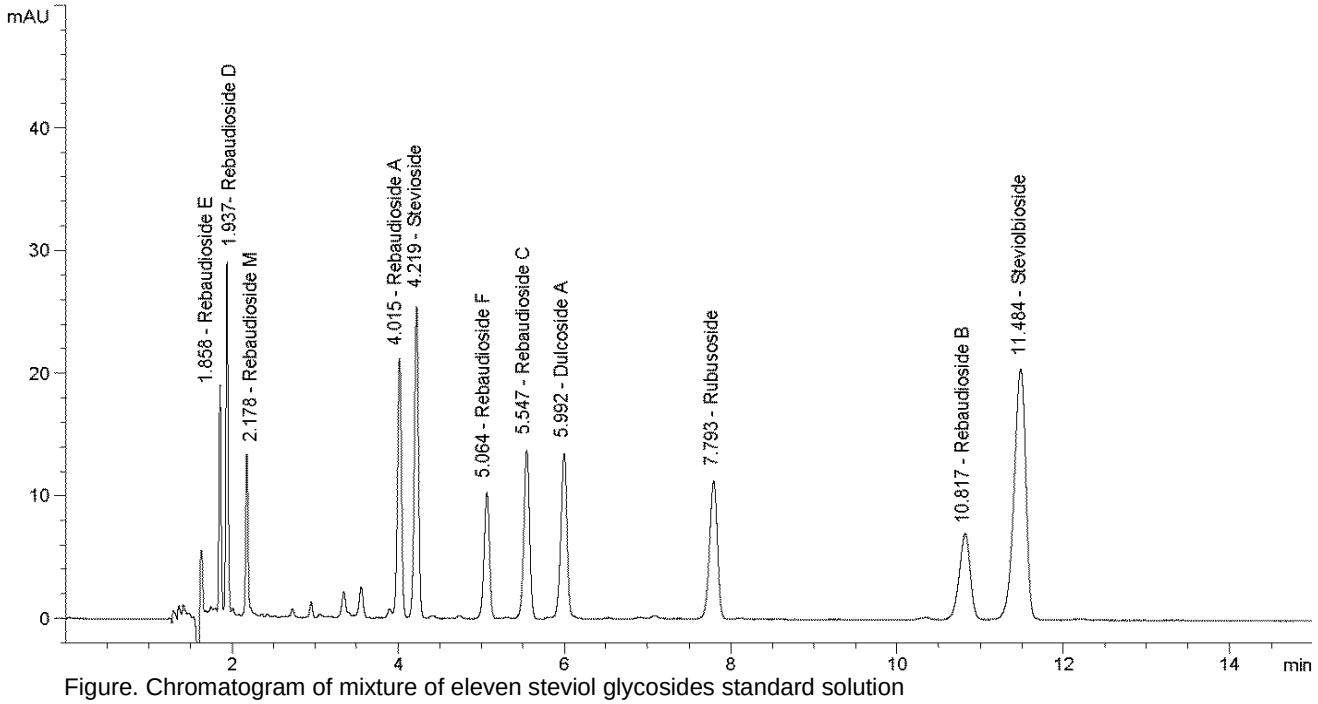


Figure. Chromatogram of mixture of eleven steviol glycosides standard solution
Column: Poroshell 120 SB-C18
Concentration: About 50mg/LB each except steviol bioside (about 100mg/L)

PARAGUAY

Name of the substance(s):	**Stevia extract, steviol glycosides (INS 960), purity 85% and 90%**
Question(s) for JECFA *(Please provide a brief justification for the request in case of re-evaluation)*	Safety assessment to consider the extension of the ADI for stevia extract, 85% - 90% purity, and inclusion of new specifications.

Rationale for the request

Stevia extracts in the market are obtained from natural sources (leaves of the Stevia rebaudiana Bertoni), and products with different degrees of purity are derived through various manufacturing processes. The composition of the extracts may vary in accordance with the agronomic conditions of the plant and between different manufacturers.

The commercial standard has an average of 85% purity, on the basis of natural leaves (without genetic modification) and non-invasive extraction processes.

Stevia is safe, both its leaves and its raw extracts, endorsed by many toxicological and pharmacological studies that have been performed. Therefore, the presence of impurities which represent 10% of the components of the leaves does not represent a danger to human health and may eventually influence the NOEL or the ADI in very low percentages (Prof. Jan Geuns, 2002).

Furthermore, the leaves of Stevia rebaudiana Bertoni could be used as well to prepare various sweet recipes to fight iron deficiency in anemia, which is an important nutritional disorder in developing countries. (Abou-Arab et. al 2010)

The industry in developed countries is presently directing the concept of Stevia extracts to glycosides, often produced with enzymatic modifications, varieties with very low agricultural strength or produced through fermentation, and away from what consumers expect from it, i.e., **what is natural.** This dangerously places Stevia extracts in an area in which they can be punished by the consumers questioning their naturalness, as well as create a broad agricultural distress and of the authorities for not achieving crops that are sustainable and scalable in a competitive way.

In line with the above, consumers in different countries (prohibition of labelling in Consumer Advice Center of Baden-Württemberg -Germany, Order of labelling in Swiss Confederation that prevents classifying products

as natural, recent Class Action to Truvia for the processing of its extract and the use of erythritol, among others) show a marked evolution towards intolerance of invasive processing with chemicals, as well as to enzymatic modifications or the results of fermentation.

Considering all of these reasons, we propose to review the possibility of extending the ADI to steviol glycosides with 85% - 90% purity.

1. Proposal for inclusion submitted by:

Instituto Nacional de Tecnologia, Normalización y Metrología (INTN) – Cámara Paraguaya de la Estevia (CAPASTE)

2. Name of the substance; commercial name(s), chemical name(s):

Stevia extract, steviol glycosides

3. Names and addresses of the basic producers:

NL Stevia SA. Ruta Gral Diaz Km 1 ½ Pirayu -Paraguay
Stevia Dolce. J. Augusto Saldivar. Paraguay
Agroindustrias de la Stevia. Km 0,7 vía Santa Elena - El Castillo - Municipio de El Cerrito - Valle del Cauca. Colombia
Ingredion Colombia SA Carrera 5 No. 52 - 56 / A.A. 6560 / PBX: (57) (2) 431 5000/
Fax: (57) (2) 431 5048
Sweet Harvest SA. BOGOTA (BOGOTA). CA 54 B 120 27 AP 302

4. Has the manufacturer committed to provide data?

Yes

5. Manufacturer that will provide data (Please submit the name of the person):

- NL STEVIA SA- Lic. Julio Cazal-Paraguay
- Stevia dolce. Jose Luis Fernandez-Paraguay
- Agroindustrias de la Stevia SA - T.A Cesar Augusto Sanchez Varela. Colombia
- Ingredion Colombia SA - Ing. Alvaro Lopez. Colombia
- Sweet Harvest SA - Ing. Juan Carlos Rodriguez. Colombia

6. Justification for use:

Intensive sweetener food ingredient

7. Food products and food categories of the General Standard for Food Additives in which this compound is used, as food additive or as ingredient; specify the levels employed:

In annex A

8. Is this compound currently used in foods that are legally in the market in more than one country? (please identify the countries); or, has the use of the compound in food been approved in more than one country? (please identify the country (countries)).

Paraguay – Bolivia – Colombia

9. List of available data (please check their availability).

Toxicological data *(i, ii, iii, were submitted for previous assessments)*

(i) Metabolic and pharmacokinetic studies. Available

(ii) Studies of short-term toxicity, carcinogenicity/long-term toxicity, reproductive toxicity and developmental toxicity in animals and studies of genotoxicity. Available

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

Available

(iv) Other data.

- Study on chemical composition and biological activities of essential oil and extracts from *Stevia rebaudiana* Bertoni leaves. Muanda *et al*. 2011
- Sweet and non-sweet constituents of *Stevia rebaudiana.* Edward J. Kennelly. Tailor&Francis. 2002

- Stevia rebaudiana Bertoni, source of a high-potency natural sweetener:

A comprehensive review on the biochemical, nutritional and functional aspects. Lemus-Mondaca *et al*. 2012. Additional information will be provided for the identification and quantification of the 10% of impurities, subject to safety assessment.

Technological data

(i) Specifications of the identity and purity of the compounds listed (specifications applied during development and toxicological studies; proposed specifications for trade). Specifications for extracts with 85% and 90% will be provided

(ii) Technological and nutrition considerations related to the manufacture and use of the compound. Pending submission

Intake assessment data

To be provided

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

a) Stevia extracts with 85% purity are used in the following food categories.

Food Cat. No	Food categories	Max Level
12.2.2	**Seasonings and condiments**	**30 mg/kg**
15.0	Ready-to-eat savouries	170 mg/kg
06.8.1	Soybean-based beverages	200 mg/kg
14.1.5	Coffee, coffee substitutes, tea, herbal infusions, and other hot cereal and grain beverages, excluding cocoa	200 mg/kg
06.3	Breakfast cereals, including rolled oats	350 mg/kg
13.6	Food supplements	2500 mg/kg
12.7	Salads (e.g. macaroni salad, potato salad) and sandwich spreads excluding cocoa-and nut-based spreads of food categories 04.2.2.5 and 05.1.3	115 mg/kg
04.2.2.8	Cooked or fried vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), and seaweeds	40 mg/kg
04.2.2.4	Canned or bottled (pasteurized) or retort pouch vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), and seaweeds	70 mg/kg
04.2.2.3	Vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), and seaweeds in vinegar, oil, brine, or soybean sauce	330 mg/kg
04.2.2.2	**Dried vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweeds, and nuts and seeds**	**40 mg/kg**

In all categories it is expressed as steviol equivalent

b) Stevia extracts of 90% purity are used in all food categories specified in the GSFA, for extracts of 95% purity.

(ii) Estimations of the intakes through foods based on data from the consumption of foods in which this compound can be used. Pending submission

Other necessary information

10. Date on which data could be submitted

June 2015

ANNEX A

STEVIOL GLYCOSIDES, 95% purity			
Food Cat. No	Food categories	Max Level	Note
12.2.2	**Seasonings and condiments**	**30 mg/kg**	
13.5	Dietetic foods (e.g.supplementary foods for dietary use) excluding products of food categories 13.1- 13.4 and 13.6	660 mg/kg	26&198
13.3	Dietetic foods intended for special medical purposes (excluding products of food category 13.1)	350 mg/kg	
15.0	Ready-to-eat savouries	170 mg/kg	
14.1.4	Water-based flavoured drinks, including "sport," "energy," or "electrolyte" drinks and particulated drinks	200 mg/kg	
06.8.1	Soybean-based beverages	200 mg/kg	
14.2.7	Aromatized alcoholic beverages (e.g beer, wine and spirituous cooler-type beverages, low-alcoholic refreshers)	200 mg/kg	
01.1.2	Dairy-based drinks, flavoured and/or fermented (e.g., chocolate milk, cocoa, eggnog, drinking yoghurt, whey-based drinks)	200 mg/kg	Note 26 & 201
14.1.5	Coffee, coffee substitutes, tea, herbal infusions and other hot cereal and grain beverages, excluding cocoa	200 mg/kg	26& 160
06.3	Breakfast cereals, including rolled oats	350 mg/kg	
13.6	Food supplements	2.500 mg/kg	26&203
04.1.2.5	Jams, jellies, marmelades	360 mg/kg	
05.2	Confectionery including hard and soft candy, nougats, etc. other than food categories 05.1, 05.3 and 05.4	700 mg/Kg	26&199
11.6	Table-top sweeteners, including those containing high-intensity sweeteners	GMP	
12.7	Salads (e.g. macaroni salad, potato salad) and sandwich spreads Excluding cocoa- and nut-based	115 mg/kg	

Spreads of food categories 04.2.2.5 and 05.1.3			
Food Cat. No	Food categories	Max Level	Note
04.1.2.12	Cooked fruit	40 mg/kg	
04.1.2.7	Candied fruit	40 mg/kg	
04.1.2.4	Canned or bottled (pasteurized) fruit	330 mg/kg	
04.1.2.3	Fruit in vinegar, oil, or brine	100 mg/kg	
05.3	Chewing gum	3,500 mg/kg	
03.0	Edible ices, including sherbet and sorbet	270 mg/kg	
04.2.2.8	Cooked or fried vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), and seaweeds	40 mg/kg	
04.2.2.4	Canned or bottled (pasteurized) or retort pouch vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), and seaweeds	70 mg/kg	
04.2.2.3	Vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), and seaweeds in vinegar, oil, brine, or soybean sauce	330 mg/kg	
04.2.2.2	Dried vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), and seaweeds and nuts and seeds	40 mg/kg	
12.6.3	Mixes for sauces and gravies	350 mg/kg	26&127
12.4	Mustards	130 mg/kg	
14.1.3	Fruit and vegetable nectars	200 mg/kg	
12.9.2.3	Other soybean sauces	165 mg/kg	
09.4	Fully preserved, including canned or fermented fish and fish products, including mollusks, crustaceans, and echinoderms	100 mg/kg	
09.3.2	Fish and fish products, including mollusks, crustaceans, and echinoderms, pickled and/or in brine	165 mg/kg	
09.3.1	Fish and fish products, including mollusks, crustaceans, and echinoderms, marinated and/or in jelly	100 mg/kg	26&144
06.5	Cereal and starch based desserts (e.g., rice pudding, tapioca pudding)	165 mg/kg	
04.1.2.9	Fruit-based desserts, incl. fruit-flavoured water-based desserts	350 mg/kg	

02.4	Fat-based desserts excluding dairy-based dessert products of food category 01.7	330 mg/kg	
10.4	Egg-based desserts (e.g., custard)	330 mg/kg	
01.7	Dairy-based desserts (e.g., pudding, fruit or flavoured yoghurt)	330 mg/kg	
04.1.2.8	Fruit preparations, including pulp, purees, fruit toppings and coconut milk	330 mg/kg	
13.4	Dietetic formulae for slimming purposes and weight reduction	270 mg/kg	
04.2.2.7	Fermented vegetable (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera) and seaweed products, excluding fermented soybean products of food categories 06.8.6, 06.8.7, 12.9.1, 12.9.2.1 and 12.9.2.3	200 mg/kg	
01.5.2	Milk and cream powder analogues	330 mg/kg	Note 26&201
08.3.2	Heat-treated processed comminuted meat, poultry, and game products	100 mg/kg	26&202
04.1.2.10	Fermented fruit products	115 mg/kg	
04.1.2.6	Fruit-based spreads (e.g., chutney) excluding products of food category 04.1.2.5	330 mg/kg	
04.2.2.6	Vegetable (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed pulps and preparations (e.g., vegetable desserts and sauces, candied vegetables) other than food category 04.2.2.5	165 mg/kg	
04.2.2.5	Vegetable (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed purees and spreads (e.g., peanut butter)	330 mg/kg	
04.1.2.11	Fruit fillings for pastries	330 mg/kg	
12.9.2.1	Fermented soybean sauce	30 mg/kg	
12.9.2.2	Non-fermented soybean sauce	165 mg/kg	
12.6.1	Emulsified sauces and dips (e.g., mayonnaise, salad dressing, onion dip)	350 mg/kg	
12.6.4	Clear sauces (e.g., fish sauce)	350 mg/kg	
12.6.2	Non-emulsified sauces (e.g., ketchup, cheese sauce, cream sauce, brown gravy)	350 mg/kg	
12.5	Soups and broths	50 mg/kg	
09.3.3	**Salmon substitutes, caviar, and other fish roe products**	**100 mg/Kg**	

Note 26: As steviol equivalents.
Note 127: As served to the consumer.
Note 144: For use in sweet and sour products only.
Note 160: For use in ready-to-drink products and pre-mixes for ready-to-drink products only.
Note 198: Use level for solid products (e.g., energy, meal replacement or fortified bars); 600 mg/kg as steviol equivalents for use in liquid products.
Note 199: For use in microsweets and breath freshening mints at 6000 mg/kg as steviol equivalents.
Note 201: For use in flavoured products only.
Note 202: For use in brine used in the production of sausage only.
Note 203: For use in chewable supplements only