European Union comments on

Codex Circular Letter CL 2013/12-FA

Priority list of substances proposed for evaluation by JECFA

*Mixed Competence.*

*Member States Vote.*

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)

**Enclosures:**

The forms containing information on the substances mentioned above.
FORM ON WHICH INFORMATION ON THE COMPOUND TO BE EVALUATED BY JECFA IS PROVIDED

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.

<table>
<thead>
<tr>
<th>Name of Compound(s):</th>
<th>Glucose oxidase from <em>Penicillium chrysogenum</em> expressed in <em>Aspergillus niger</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Question(s) to be answered by JECFA</td>
<td>Safety evaluation when used as processing aid.</td>
</tr>
<tr>
<td>(kindly provide a brief justification of the request in case of re-evaluations)</td>
<td></td>
</tr>
</tbody>
</table>

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
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
- France : 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. 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.

**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)

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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.

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.
- 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 human 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.
FORM ON WHICH INFORMATION ON THE COMPOUND TO BE EVALUATED BY JECFA IS PROVIDED

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.

<table>
<thead>
<tr>
<th>Name of Compound(s):</th>
<th>Asparaginase from Aspergillus niger expressing a modified gene from Aspergillus niger</th>
</tr>
</thead>
<tbody>
<tr>
<td>Question(s) to be answered by JECFA (kindly provide a brief justification of the request in case of re-evaluations)</td>
<td>Safety evaluation when used as processing aid.</td>
</tr>
</tbody>
</table>

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 Cèdex
   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
6. Justification for use:

Acrylamide, which is an 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. 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.

**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)

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.
• 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 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.
FORM ON WHICH INFORMATION ON THE COMPOUND TO BE EVALUATED BY JECFA IS PROVIDED

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.

<table>
<thead>
<tr>
<th>Name of Compound(s):</th>
<th>Phospholipase A₂ from pig pancreas expressed in Aspergillus niger</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Question(s) to be answered by JECFA</strong></td>
<td>Safety evaluation when used as processing aid.</td>
</tr>
<tr>
<td>(kindly provide a brief justification of the request in case of re-evaluations)</td>
<td></td>
</tr>
</tbody>
</table>

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 A₂ from pig pancreas expressed in *Aspergillus niger*
- **Trade names**: Maxapal A₂, Cakezyme smart, Purifine SB2, Purifine SB3, Purifine SB4, Purifine RS1, Purifine CN1
- **Chemical names**: Phospholipase A₂ (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
6. Justification for use:

The enzyme phospholipase A2 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
- 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. 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.

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

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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 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, \textit{in vivo}
- Human lymphocyte cytogenetic assay (\textit{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 \textit{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.

\textbf{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 \textit{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\(^\circ\)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.

\textbf{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 118 mg 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.
GSC CODEX MESSAGE CCFA46/2014/37

FORM ON WHICH INFORMATION ON THE COMPOUND TO BE EVALUATED BY JECFA IS PROVIDED

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.

<table>
<thead>
<tr>
<th>Name of Compound(s):</th>
<th>INS 392 Rosemary extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Question(s) to be answered by JECFA</td>
<td>Safety assessment when used as a food additive (antioxidant)</td>
</tr>
<tr>
<td>(kindly provide a brief justification of the request in case of re-evaluations)</td>
<td></td>
</tr>
</tbody>
</table>

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>
<thead>
<tr>
<th>Food Category*</th>
<th>Sub-category</th>
<th>Use level (mg/kg)*</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 Dairy products and analogues</td>
<td>1.5.1 Milk powder and cream powder (plain)</td>
<td>30</td>
<td>Dried milk for the manufacturing of ice cream</td>
</tr>
<tr>
<td></td>
<td></td>
<td>200*</td>
<td>Milk powder for vending machines</td>
</tr>
<tr>
<td>2.0 Fats and oils and fat emulsions</td>
<td>2.1.3 Lard, tallow, fish oil and other animal fats</td>
<td>50*</td>
<td>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</td>
</tr>
<tr>
<td></td>
<td>2.1.2 Vegetable oils and fats</td>
<td>30*</td>
<td>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</td>
</tr>
<tr>
<td></td>
<td>2.2.2 Fat spreads, dairy fat spreads and blended spreads</td>
<td>30*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.1.2 Vegetable oils and fats</td>
<td>50*</td>
<td>Only fats and oils for the professional manufacture of heat treated foods</td>
</tr>
<tr>
<td>4.0 Fruit and vegetables, seaweed and nuts and seeds</td>
<td>4.2.2.6 Vegetable, seaweed and nut and seed pulps and preparations other than food category 4.2.2.5</td>
<td>200</td>
<td>Only seaweed based fish roe analogues</td>
</tr>
<tr>
<td></td>
<td>4.2.2.5 Vegetable, seaweed and nut and seed purees and spreads (e.g. peanut butter)</td>
<td>200*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.2.2.2 Dried vegetables, seaweeds and nuts and seeds</td>
<td>200</td>
<td>Dehydrated potato products</td>
</tr>
<tr>
<td>5.0 Confectionery</td>
<td>5.3 Chewing gum</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.4 Decorations, toppings (non-fruit) and sweet sauces</td>
<td>100*</td>
<td>Sauces only</td>
</tr>
<tr>
<td>7.0 Bakery wares</td>
<td>7.2 Fine bakery wares and mixes</td>
<td>200*</td>
<td></td>
</tr>
<tr>
<td>8.0 Meat and meat products, including poultry and game</td>
<td>8.2.1 Non heat treated processed meat, poultry and game products in whole pieces or cuts</td>
<td>15*</td>
<td>Only meat with a fat content not higher than 10%, excluding dried sausages</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150*</td>
<td>Only meat with a fat content higher than 10%, excluding dried sausages</td>
</tr>
<tr>
<td>Food Category“</td>
<td>Sub-category</td>
<td>Use level (mg/kg)“</td>
<td>Notes</td>
</tr>
<tr>
<td>---------------</td>
<td>--------------</td>
<td>--------------------</td>
<td>-------</td>
</tr>
<tr>
<td>8.3.1 Non heat treated processed comminuted meat, poultry and game products</td>
<td>100</td>
<td>Only dried sausages</td>
<td></td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>Only dehydrated meat</td>
<td></td>
</tr>
<tr>
<td>8.2.2 Heat treated processed meat, poultry and game products in whole pieces or cuts</td>
<td>15*</td>
<td>Only meat with a fat content not higher than 10%, excluding dried sausages</td>
<td></td>
</tr>
<tr>
<td></td>
<td>150*</td>
<td>Only meat with a fat content higher than 10%, excluding dried sausages</td>
<td></td>
</tr>
<tr>
<td>8.3.2 Heat treated processed comminuted meat, poultry and game products</td>
<td>100</td>
<td>Only dehydrated sausages</td>
<td></td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>Only dehydrated meat</td>
<td></td>
</tr>
<tr>
<td>9.0 Fish and fish products, including mollusks, crustaceans and echinoderms</td>
<td>9.2 Processed fish and fishery products, including mollusks, crustaceans and echinoderms</td>
<td>15</td>
<td>Only fish and fishery products, including mollusks and crustaceans with a fat content not higher than 10%</td>
</tr>
<tr>
<td></td>
<td>150*</td>
<td>Only fish and fishery products, including mollusks and crustaceans with a fat content higher than 10%</td>
<td></td>
</tr>
<tr>
<td>10.0 Eggs and egg products</td>
<td>10.2 Egg products</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>12.0 Salts, spices, soups, sauces, salads, protein products</td>
<td>12.2.2 Seasonings and condiments</td>
<td>200*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.4 Mustard s</td>
<td>100*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.5 Soups and broths</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.6 Sauces and like products</td>
<td>100*</td>
<td></td>
</tr>
<tr>
<td>15.0 Ready-to-eat savouries</td>
<td>15.1 Snacks - potato, cereal, flour, or starch-based</td>
<td>50*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15.2 Processed nuts, including coated nuts and nut mixtures</td>
<td>200*</td>
<td></td>
</tr>
<tr>
<td>13.0 Foodstuffs intended for particular nutritional uses</td>
<td>13.6 Food supplements</td>
<td>400</td>
<td></td>
</tr>
</tbody>
</table>

“Use level expressed on fat basis
“ Grouped in food uses categories according to the Codex GSFA food categorisation system for food additives http://www.codexalimentarius.net/gsfaonline/index.html
“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


Please refer to the reference list of this document.
FORM ON WHICH INFORMATION ON THE COMPOUND TO BE EVALUATED BY JECFA IS PROVIDED

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.

<table>
<thead>
<tr>
<th>Name of Compound(s):</th>
<th>Xylanase from <em>Talaromyces emersonii</em> expressed in <em>Aspergillus niger</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Question(s) to be answered by JECFA</strong>&lt;br&gt; (kindly provide a brief justification of the request in case of re-evaluations)</td>
<td>Safety evaluation when used as processing aid.</td>
</tr>
</tbody>
</table>

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\(^1\). 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\(^2\).

**Toxicological data**

(i) Metabolic and pharmacokinetic studies

Not applicable.

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\(^1\) 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.

(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)

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/1 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.