Annex 3

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.

Name of Compound(s):	**Acid prolyl endopeptidase from a genetically modified strain of *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 : Acid prolyl endopeptidase from a genetically modified strain of *Aspergillus niger*

Trade names : BREWERS CLAREX®, MAXIPRO PSP

Chemical name : Acid prolyl endopeptidase (EC 3.4.21.xx[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 Jack Reuvers

[1] The name proline endopeptidase is also a synonym of prolyl oligopeptidase, EC (IUBMB) number 3.4.21.26. However, in contrast to oligopeptidases, the enzyme also acts on proteins (Edens *et al.*, 2005; Kubota *et al.*, 2005; Takahashi, 2013).

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DSM Food Specialties
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6. Justification for use:

The enzyme preparation is used in beer brewing, potable alcohol production, protein processing and starch processing to catalyze the cleavage of peptide bonds in proteins and peptides, mainly at the carboxylic site of proline residues. The technological need of the enzymatic conversion of proteins and peptides with the help of acid prolyl endopeptidase in these applications can be described as:

- Brewing: degradation of the substrate (proteins and peptides) which otherwise may cause haze in the final product, and to reduce the amount of gluten (gliadins);
- Potable alcohol production: creation of reaction products (smaller peptides) for optimal development of the fermentation;
- Protein processing: degradation of the substrate (proteins and peptides) to obtain a protein hydrolysate without bitter taste;
- Starch processing: degradation of the substrate (proteins and peptides) which would have otherwise a negative influence on the production process, and to reduce the amount of gluten (gliadins) which otherwise might end up in the processed starch product.

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, potable alcohol production, protein processing and starch processing in accordance with current Good Manufacturing Practice (cGMP). The dosage of the enzyme varies depending on the specific application:

- Brewing: between 0.7 and 2.1 mg Total Organic Solids (TOS)/kg wort
- Potable alcohol production: between 70 and 704 mg Total Organic Solids (TOS)/kg cereals (dry matter)
- Starch processing: between 0.21 and 3.5 mg Total Organic Solids (TOS)/kg liquefied starch
- Protein processing: between 10000 and 15000 Total Organic Solids (TOS)/kg protein (dry matter)

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 acid prolyl endopeptidase derived from a genetically modified strain of *Aspergillus niger* is authorized in the following countries:

- Australia/New Zealand : Food Standard 1.3.3 on Processing Aids
- Brazil : Diário Oficial da União 2009

- China : Food Safety National Standards for the Usage of Food Additives, GB 2760-2011
- France : Afssa – Saisine n° 2005-SA-0002
- Denmark : Certificate number 2005-20-5406-00080/IM
- Mexico : Diario Oficial 2012 (Anexo VI)
- Russia : Certificate number RU.77.99.26.009.E.005410.03.11

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

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):

- Test for mutagenic activity (Ames test)
- Chromosomal aberration test (*in vitro*)
- 90-days oral toxicity study in rats

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

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

90-days oral administration of the enzyme to rats did not cause in dose related findings. Therefore, the highest dose administered, 5040 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.

[2] 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.

[3] 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

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, 9th 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 beer brewing, potable alcohol production, protein processing and starch processing. The action of the enzyme present in the preparation takes place in a particular process step depending on the application.

In brewing, the food enzyme is typically added to the cooled wort at the beginning of the fermentation step. During pasteurization, acid prolyl endopeptidase will be party denatured. Due to depletion of the substrate, the remaining non-denatured enzyme molecules will not exert a technological function in the final brewing product, just like other enzymes that are produced by the brewers yeast and able to partly survive the pasteurization step.

In potable alcohol production, the food enzyme is added during the pre-saccharification and fermentation steps. During the distillation process, the enzyme proteins are completely removed. Consequently, no acid prolyl endopeptidase activity will be present in the final potable alcohol.

In protein processing, the food enzyme is typically added at the very beginning of the hydrolysis process. During the sterilization step, the enzyme protein is denatured.

In starch processing, the food enzyme is typically added during the saccharification step. During the heating steps, the enzyme protein is denatured.

Taken together, no residual enzyme activity remains in the final product of all applications. 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.

- Brewing: based on the dose of 0.7- 2.1 mg TOS/kg wort and the fact that 1 L wort results in 1 L, the amount of TOS in the final product will be 0.7- 2.1 mg TOS/ L beer.

- Potable alcohol production: based on the fact that during the distillation process all the TOS will be eliminated, it is assumed that nothing of the TOS will end up in the final product

- Starch processing for beverages: based on the dose of 0.21-3.5 mg TOS/kg liquefied starch and assuming that the ratio from starch hydrolysate to glucose syrup is 0.93 and the ratio from glucose syrup to soft drinks is 0.2, the total ratio will be 0.93x0.2=0.186 and therefore the amount of TOS in the final product will be 0.04-0.65 mg TOS/L soft drink

- Starch processing for solid foods: based on the dose of 0.21-3.5 mg TOS/kg liquefied starch and assuming that the ratio from starch hydrolysate to glucose syrup is 0.93 and the ratio from glucose syrup to confectionery (including candies) is 0.7, the total ratio will be 0.93x0.7=0.65 and

therefore the amount of TOS in the final product will be 0.14-2.28 mg TOS/kg jams, biscuits, confectionery, ice cream, etc.

- Protein processing: based on the dose of 10000-15000 TOS/kg protein (dry matter) and assuming that the maximal dose of protein hydrolysates in food is 8.5%, the amount of TOS in the final product will be 850-1275 mg TOS/kg soups, protein shakes, dressings, etc.

(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, assuming that the daily intake of processed foods is 50% of the total solid food intake, i.e. 0.0125 kg/kg bw/day and that the daily intake of soft drinks (assuming that beer is consumed in the same amount as soft drinks) is 25% of the total beverages intake, i.e. 0.025 l/ kg bw/day, and calculating on basis of the **maximal** values found in food and beverage (in the above cases beer and foods containing protein hydrolysates), the total daily intake will be 10.64– 16.0 mg TOS/kg bw/day.

Other information as necessary

None.

10. Date on which data could be submitted to JECFA

As soon as necessary.