

FORM FOR THE SUBMISSION OF SUBSTANCES TO BE EVALUATED BY JECFA

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

Name of Substance(s):	Inulinase from <i>Aspergillus ficuum</i> produced by <i>Aspergillus oryzae</i> , strain MUCL 44346
Question(s) to be answered by JECFA	Safety evaluation when used as a processing aid

1. Proposal for inclusion submitted by:

The Belgian Federal Public Service for Health, Food Chain Safety and Environment
Place Victor Horta 40 box 10
1060 Brussels/Sint-Gillis
Belgium

E-mail: apf.food@health.belgium.be

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

Name: Inulinase from *Aspergillus ficuum* produced by *A. oryzae*, strain MUCL 44346
IUBMB name: EC 3.2.1.7
Tradename: Oligofruct'ase (Main commercial name)
Chemical names: Inulase; indoinulinase; endo-inulinase; exoinulinase; 2,1-β-D-fructan fructanohydrolase

3. Names and addresses of basic producers

Puratos NV- site Beldem (Andenne)
Rue Bourrie 12
B-5300 Andenne
Belgium
Tel. no: +32 8582 3250
Fax no: +32 8582 3260

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

Puratos NV commits to provide data to support the proposal for the inclusion of Inulinase from *A. ficuum* produced by *A. oryzae* in the list of substances to be evaluated by the JECFA.

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

Name: Puratos NV
Address: Industrialaan 25
Postal code and City: B-1702 Groot-Bijgaarden
Country: Belgium
Tel. no: +32 2481 4444
Fax no: +32 2466 2581
Contact person: Bas Verhagen
Tel: +32 2481 4551
E-mail: BVerhagen@puratos.com

6. Justification for use:

The food enzyme catalyses the hydrolysis of inulin to produce fructo-oligosaccharides. In principle, the enzymatic conversion of inulin with the help of inulinase may be used in the processing of all food raw materials which naturally contain inulin.

The food enzyme object of this dossier is typically used in the following food manufacturing processes:

- production of fructo-oligosaccharides

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 enzyme preparation is used as a processing aid during production of fructo-oligosaccharides. The effect of the enzymatic conversion with the help of inulinase is the hydrolysis of (2→1)-β-D-fructosidic linkages in inulin to produce fructo-oligosaccharides (FOS). This FOS is a quite pure product which can be used as sweetener to replace sucrose or as dietary fibre. The food enzyme is denatured during the FOS processing.

Food enzyme preparations are used by food manufacturers according to the *Quantum Satis* principle, which means that food manufacturers will typically fine-tune the enzyme dosage based on a dose range recommended by the enzyme supplier. The recommended dose ranges for this inulase is 1000 - 2750 IU per kg raw material (Inulin).

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

The use in food of the inulinase from *A. ficuum* produced *A. oryzae* is officially approved in France and the USA.

Moreover, the enzyme is legally traded and the use in food is legal in many countries worldwide, including in the EU.

Finally, a new dossier has also been submitted in the EU under Regulation (EC) No 1332/2008, and is currently under review by the EFSA.

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

Toxicological data

A. oryzae is used since nearly 500 years in Japan to produce "Koji". The Koji is one of the basis of the traditional foods in this country. JECFA recommends testing food enzymes derived from fungal origin for the presence of the secondary metabolites. Based on the JECFA recommendations, already since decades a wide variety of food enzymes from *Aspergillus* sp. have been tested for their safety as well as presence of potential unsafe secondary metabolites. *A. oryzae* does not produce aflatoxins, as was confirmed by the absence of mycotoxins in production batches.

The US EPA has exempted *A. oryzae* from review by the Agency, due to its extensive history of safe use. Due to difficulty with the identification of *A. oryzae* and the possibility of some strains to produce certain mycotoxins, *A. oryzae* does not qualify for general QPS status by the EFSA. The EFSA does recognize that *A. oryzae* has a long history of safe use, both in food outside Europe, and for enzyme / protein production.

- (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 food enzyme has been subjected to a standard package of toxicological tests in line with internationally accepted guidelines (OECD/EU), with the following results:

Ames test:	No mutagenic activity under the given test conditions
Chromosome aberration test:	No clastogenic activity under the given test conditions
90-day oral toxicity on rats:	The No Observed Adverse Effect Level (NOAEL) is 189.65 mg TOS/kg bw/day, which is the highest dose in the study.

In short, the food enzyme object of the present dossier was subjected to several toxicological studies to confirm its safety for consumers. The mutagenicity studies supported that the food enzyme does not have the potential to damage the genetic material of living organisms,

including mammals. The oral toxicity study showed that the food enzyme does not exhibit signs of toxicity, up to doses that are thousand times higher than those which are consumed via food.

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

Not applicable

- (iv) Other data

None

Technological data

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

The food enzyme inulinase complies with the internationally accepted JECFA specifications for chemical and microbiological purity of food enzymes (FAO/WHO Monographs 3, 2006). Neither the production strain nor antibiotic resistance genes are present in the final product. Moreover, no presence of biologically active DNA has been shown.

The inulinase described in this dossier is manufactured in accordance with current Good Manufacturing Practice for Food (GMP) and the principals of Hazard Analysis of Critical Control Points (HACCP) and in line with Food Hygiene Regulation (EC) No 852/2004 and Regulation (EC) No 178/2002.

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

Inulinase from *A. oryzae* is a protein used at very low dosage. Therefore, it has no nutritional impact on final products. The food enzyme is denatured during processing. As other food proteins, the enzyme may be hydrolyzed into its constitutive amino acids. The reaction product of the hydrolysis of inulin with the help of endo-inulinase is a syrup of FOS. Like the substrate and the enzyme, the FOS also naturally occur in various organisms, including fruits. The use of inulinase from *A. oryzae* in the creation of FOS doesn't additionally alter the nutritional composition nor modify nutritional value of the final foods in which this FOS is used.

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 food enzyme object of this dossier is typically used in the production of fructo-oligosaccharides.

Based on the maximum recommended use level for the enzyme per raw material (inulin), the maximum level of TOS in the FOS will be 20.86 mg TOS/ kg food.

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

The total Theoretical Maximum Daily Intake (TMDI) can be calculated on basis of the maximal values found in foods, multiplied by the maximum consumption of foods/kg body weight/day. Based on the recommended use levels, the amounts of the respective ingredients (FOS) that end up in the final foods (e.g. confectionary, fine bakery ware) and the amount of the wide range of possible foodstuffs with FOS consumed by the high end consumers, the TMDI of the food enzyme inulinase from *A. oryzae* was calculated to be 0.0069 mg TOS/kg body weight/day.

It should be stressed that this total TMDI is based on conservative assumptions and represents a highly exaggerated value.

Other information (as necessary/identified)

None

As soon as required.