CODEX ALIMENTARIUS COMMISSION

Food and Agriculture Organization of the United Nations

World Health Organization

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Agenda Item 8 (a) **CX/FA 13/45/16 Add.1**

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JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON FOOD ADDITIVES

Forty-fifth Session

Beijing, China 18-22 March 2013

PROPOSALS FOR ADDITIONS AND CHANGES TO THE PRIORITY LIST OF FOOD ADDITIVES PROPOSED FOR EVALUATION BY JECFA

(Replies to CL 2012/8-FA)

The following comments have been received from the following Codex members and observers

European Union

EUROPEAN UNION

In addition to the compounds proposed by the European Union and its Member States (see CX/FA 13/45/16) the EUMS are proposing to add also the following compounds to the priority list of compounds to be proposed for evaluation by JECFA:

1) **Lipase from *Fusarium heterosporum* expressed in *Hansenula polymorpha***
2) **Maltotetraohydrolase from *Bacillus licheniformis* expressing a modified Maltotetraohydrolase Gene from *Pseudomonas saccharophila***

The forms containing information on the compounds to be evaluated by JECFA are attached.

The EUMS apologise for any inconvenience caused by the late submission of these additional proposals.

Appendix 1. Lipase from *Fusarium heterosporum* expressed in *Hansenula polymorpha*

Name of Compound(s):	**Lipase from *Fusarium heterosporum* expressed in *Hansenula polymorpha***
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: Lipase from *Fusarium heterosporum* expressed in *Hansenula polymorpha*

Trade names: GRINDAMYL CAPTIVE, POWERBAKE and HPL KLM1 (main commercial names).

Chemical names: lipase (EC 3.1.1.3)

3. Names and addresses of basic producers:

DuPont Industrial Biosciences (Danisco US Inc.)
925 Page Mill Road
Palo Alto, CA 94304
UNITED STATES
Tel.: +1 650 846 7500

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

DuPont Industrial Biosciences (Danisco US Inc.) commits to provide data to support the proposal for inclusion of the lipase in the list of substances to be evaluated by JECFA.

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

DuPont Industrial Biosciences (Danisco US Inc.)
925 Page Mill Road
Palo Alto, CA 94304
UNITED STATES
Tel.: +1 650 846 7500

Attn.: Fred Wondergem Drs., Regulatory Affairs Manager

fred.wondergem@dupont.com

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6. Justification for use:

The enzyme preparation is used in bread and noodle dough to modify the endogenous lipids. In bread this improves dough stability and dough handling properties, improves bread volume and creates a homogenous crumb structure. In noodles the quality of the finish product is improved with less cooking loss and improved eating quality. The enzyme preparation can also be used in egg yolk to modify phospholipids for use in cake manufacture and mayonnaise. Finally the enzyme preparation can be used in oil degumming to hydrolyse phospholipids to lyso-phospholipids.

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 bread, pasta and noodles, egg yolk and oil degumming in accordance with current Good Manufacturing Practices (cGMP). The dosage of the enzyme varies between 4.46 and 178 mg Total Organic Solids (TOS)/kg flour for bakery and noodle applications. The dosage of the enzyme varies between 11.2 and 892 mg Total Organic Solids (TOS)/kg egg yolk for egg yolk applications and finally the dosage of the enzyme varies between 8.9 and 89 mg Total Organic Solids (TOS)/kg crude oil in oil degumming applications.

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 lipase from *Fusarium heterosporum* expressed in *Hansenula polymorpha* is authorized in the following countries:

- Denmark: Ministry of Food, Agriculture and Fisheries, Danish Veterinary and Food Administration, File No 2005-20-5406-00075 (March 2, 2007)
- France: Arrete 2006
- USA: GRAS Notice 238 http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=238
- Australia: Food Standard 1.3.3 on Processing Aids
- Mexico: Dairio Oficial 2012

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

The production organism is from a safe strain as described in the decision tree in Pariza and Johnson, 2001[1]. However, to accommodate various registration requirements in different countries world-wide, a full

[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.

toxicity program for food enzymes has been performed according to the SCF 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)
- Human lymphocyte cytogenetic assay (*in vitro* micronucleus test)
- 13 weeks oral toxicity study in rats

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

The enzyme from *Fusarium heterosporum* expressed in *Hansenula polymorpha* 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, 669 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 lipase enzyme preparation from *Hansenula polymorpha* will be used as a processing aid in the manufacture of bakery products, pasta and noodles, egg yolk and oil degumming. The action of the enzyme takes place in the bread and noodle dough. The enzyme activity will be lost during the baking and cooking step, respectively. The enzyme preparation will be added to the egg yolk before the pasteurization step in which the enzyme activity will be lost. The enzyme will be removed from the refined oil during the separation of the water and oil phase as the enzyme will be dissolved in the water phase. No residual enzyme activity remains in the final product after use in the above 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

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

Application	Raw material (RM)	Minimal use level (mg TOS/ kg RM)	Maximal use level (mg TOS/ kg RM)	Final food	Ratio RM/ final food	Minimal level in final food (mg TOS/ kg final food)	Maximal level in final food (mg TOS/ kg food)
Egg yolk	Egg yolk	11,2	892	Cake/ Mayonnaise	0,15	1,68	133,8
Oil degumming	Oil	8,9	89	Oil	1	8,90	89
Noodles	Flour	4,46	178,4	Noodles	0,75	3,35	133,8
Baking	Flour	4,46	178,4	Bread	0,71	3,17	126,7

(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 using noodles as the worst case scenario, the daily intake will be 3.35 – 418 µg TOS/kg bw/day.

Other information as necessary

None

10. Date on which data could be submitted to JECFA

As soon as necessary.

Appendix 2. Maltotetraohydrolase from *Bacillus licheniformis* expressing a modified Maltotetraohydrolase Gene from *Pseudomonas saccharophila*

Name of Compound(s):	**Maltotetraohydrolase from *Bacillus licheniformis* expressing a modified Maltotetraohydrolase Gene from *Pseudomonas saccharophila***
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: Maltotetraohydrolase from *Bacillus licheniformis* expressing a modified Maltotetraohydrolase gene from *Pseudomonas saccharophila*

Trade names: GRINDAMYL, POWERFresh, POWERFlex (main commercial names)

(Various other commercial names will be used, like GRINDAMYL CAPTIVE 2144, GRINDAMYL POWERFresh 5002, POWERFlex 6001 etc.)

Chemical names: EC 3.2.1.60 and CAS number 37288-44-1

3. Names and addresses of basic producers:

DuPont Industrial Biosciences (Danisco US Inc.)
925 Page Mill Road
Palo Alto, CA 94304
UNITED STATES
Tel.: +1 650 846 7500

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

DuPont Industrial Biosciences (Danisco US Inc.) commits to provide data to support the proposal for inclusion of the maltotetraohydrolase in the list of substances to be evaluated by JECFA.

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

DuPont Industrial Biosciences (Danisco US Inc.)
925 Page Mill Road
Palo Alto, CA 94304
UNITED STATES
Tel.: +1 650 846 7500

Attn.: Fred Wondergem Drs., Regulatory Affairs Manager

fred.wondergem@dupont.com

+31 (0)71 5685 108

6. Justification for use:

The enzyme catalyses hydrolysis of 1,4-α-D-glucosidic linkages in amylaceous polysaccharides to remove successive maltotetraose residues from the non-reducing chain ends.

Maltotetraohydrolase will be used to delay the staling process in bread and other baked goods and thereby secure the freshness throughout the entire shelf-life, the products will thus have an acceptable eating quality throughout the entire shelf-life. This is a benefit to the bread producers and to consumers as bread will keep fresh during the entire shelf-life and disposal because of staling is minimized. The ability of maltotetraohydrolase to form maltotetraose can also be used in the starch processing industry. In all these applications the enzyme is considered a processing aid.

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 will be used in bakery products such as bread, bread buns, tortillas and crackers to delay the staling of these bakery products. To obtain the desired anti-staling effects of this maltotetraohydrolase, the recommended dose is 2.0-30.0 mg enzyme protein/kg flour (2.28 – 34.2 mg TOS/kg flour) in accordance with current Good Manufacturing Practices (cGMPs)

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 maltotetraohydrolase produced with this production organism is approved in the following countries:

- Denmark : Ministry of Food, Argriculture and Fisheries, Danish Veterinary and Food; Administration, File No 2009-20-5406-00013 (April 26, 2010).
- France: Arrete 2006
- USA: Department of Health & Human Services, Public Health Service, Food and Drug Administration, GRN 000277 (January 13, 2009). http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=277
- Australia: Food Standard 1.3.3 on Processing Aids
- Mexico: Approved as of June 4, 2010
- Canada: IMA published on p. 345 March 6, 2010

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 SCF 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

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

- Acute dermal irritation study in rabbits (sequential approach)
- Acute Eye Irritation/Corrosion Study in the Rabbit
- Acute oral toxicity in rats – Fixed dose procedure
- Bacterial Reverse Mutation Assay – Ames assay
- In vitro chromosomal aberration Study
- Sub-chronic 13 week toxicity in the rat

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

According to the Directive of the Commission 93/21/EEC of April 27, 1993, maltotetraohydrolase is non hazardous based on acute oral and irritation studies. In genotoxicity studies, maltotetraohydrolase is not mutagenic, clastogenic or aneugenic. Daily oral administration of maltotetraohydrolase up to and including a dose level of 79 mg total protein/kg bw/day (90.0 mg TOS/kg bw/day) does not result in any manifestation of systemic, hematologic, functional or histopathologic adverse effects and 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

Maltotetraohydrolase has been developed to retard the staling process of baked goods. It improves the softness, crumb structure and strength, and thus provides freshness through the entire shelf life, making this maltotetraohydrolase far better suitable for anti-staling application than the wild-type maltotetraohydrolase enzyme from Pseudomonas saccharophila. This is a benefit to the bread producers and to consumers as bread will keep fresh during the entire shelf-life and disposal of stale bread and baked is minimized.

Maltotetraohydrolase is a protein and any residual amounts remaining in food consumed would have the same nutritional value accordingly. However, the use levels of maltotetraohydrolase are very low. As with

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

other enzymes that are currently approved and used as processing aids, use of this product would have an insignificant impact on the nutritional value of the food.

Intake assessment data

(i) Levels of the listed 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.

To obtain the desired anti-staling effects of this maltotetraohydrolase, the recommended dose is 2.0-30.0 mg enzyme protein/kg flour (2.28 – 34.2 mg TOS/kg flour). The enzyme is largely inactivated during baking and has no further technical effect after baking

Application	**Raw material (RM)**	**Minimal use level (mg/kg RM)**	**Maximal use level (mg/kg RM)**	**Final food**	**Ratio RM/ final food**	**Minimal level in final food (mg/kg food)**	**Maximal level in final food (mg/kg food)**
Baking (enzyme protein)	Flour	2.0	30.0	Bread	0.71	1.42	21.3
Baking (TOS)	Flour	2.28	34.2	Bread	0.71	1.62	24.28

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

Assuming that 100% of enzyme remains in the bread, not removed or inactivated by heat and also on the assumption of 100% market penetration, the maximum daily exposure is 0.067 mg active enzyme protein/kg body weight/day or 0.078 mg TOS/kg body weight/day.

Other information as necessary

None

10. Date on which data could be submitted to JECFA

As soon as necessary.