ANSES opinion on the enzyme evaluation (05 Jul 2013) Courtesy Translation

Reference : Opinion on the application for authorization to use a lysophospholipase (EC 3.1.15) from a *Trichoderma reesei* carrying a gene from *Aspergillus nishimurae* for the glucose syrup production.

Application # 2012-SA-0259

National Agency for Food Safety, Environmental and Occupational Health and Safety (ANSES) was asked on 4 December 2012 by the Directorate-General for Competition, Consumption and Fraud control (DGCCRF) to risk assess the use of a use a lysophospholipase (EC 3.1.15) from a *Trichoderma reesei* carrying a gene from *Aspergillus nishimurae* for the glucose syrup production.

1. BACKGROUND

This application falls within the scope of the Decree of 10 May 2011 laying down the conditions of authorization and use of processing aids which can be used in the manufacture of foodstuffs for human consumption.

According to Article 1 of the Order of 7 March 2011, a dossier must be established according to the EFSA Guideline for the submission of a dossier on food enzymes.

2. ORGANIZATION OF EXPERTISE

The expertise was carried out in compliance with the standard NF X 50-110 "Quality in expertise-General competence requirements for an expert opinion (May 2003) ".

After the meetings of the Biotechnology Expert Committee on 21 February and 21 March 2013, ANSES made a request for additional information to the DGCCRF. On 29 April and 31 May 2013, it received some additional data allowing ANSES to continue the expertise.

The collective expertise was carried out by the Biotechnology Expert Committee on 21 February, 21 March, 16 May and 20 June 2013.

3. ANALYSIS AND CONCLUSIONS OF THE COMMITTEE

The enzyme is a lysophospholipase (E.C. 3.1.1.5, CAS 9001-85-8). It hydrolyzes phospholipids releasing one fatty acid and glycerophosphate by cleavage of an ester bond. This enzyme belongs to the group of carboxylic ester hydrolysases.

One unit of lysophospholipase activity (LPL U) is defined as the amount of enzyme necessary to produce, from a 0.01 M solution of lysolecithin, 1 µmol of fat per minute at 55 ° C and pH 4.5. The characteristics of the food enzyme are described. The protein is composed of two N-glycosylated subunits. Total organic solids (TOS) are calculated according to the formula TOS = 100% - moisture - ash - diluents. The final formulation of the lysophospholipase is in liquid form (the form recommended by the petitioner for this application) with a guaranteed minimum activity of 10000 LPL U / g and a

TOS of 1.56% (w / w). The applicant also mentions a powder form without its specifications, though.

Alpha-amylase, amyloglucosidase, beta-glucanase, cellulase, protease and xylanase side activities are indicated by the applicant.

The chemical and biological purity criteria of the food enzyme comply with the requirements of the Order of 19 October 2006 as amended. Absence of production strain and antibacterial activity in the food enzyme is substantiated.

3.2 Production organism and manufacturing process

3.2.1 Production organism

Safety of the host microorganism

Trichoderma reesei host strain is derived from the QM6a origin strain by mutagenesis and genetic transformation. *Trichoderma reesei*, a nonpathogenic and non-toxigenic organism, has a history of use for the production of enzymes.

Identity of donor microorganisms

The coding sequence of lysophospholipase was isolated from *Aspergillus nishimurae*, initially classified as *Aspergillus fumigatus.*

The coding sequence of an antibiotic resistance gene of the phleomycin family (gene *ble*) was isolated from a strain of *Streptoalloteichus hindustanus*.

Production strain

The transgenes are integrated into the fungal genome. Information is presented on the different stages of the genealogy and transformation of the production strain.

The stability of the production strain is documented by the applicant.

The production strain of the enzymatic preparation is the genetically modified *Trichoderma reesei* strain RF 7206 (RH31920 or CBS 125079). The strain selection is produced on resistance to antibiotics from the phleomycin family. The use of this antibiotic resistance for the selection of the transformants in those conditions is not of safety concerns as:

- the *ble* gene is stably integrated into the genome of the production strain
- the manufacturing process leads to the absence of the production strain and its DNA in the food enzyme,
- those antibiotics are not used for human antibiotic therapy.

3.2.2 Manufacturing process

The manufacturing process of the food enzyme is an aerobic submerged fermentation process, followed by steps of separation of the micro-organism, concentrations, filtration and enzyme formulation. Additives and processing aids used in this production are indicated and their safety is documented.

The food enzyme is produced according to Good Manufacturing Practices (cGMP) using HACCP analysis. The production plant is certified to ISO 9001: 2008 and ISO 22000: 2005. The raw materials used are food grade.

3.3. Reaction and Fate in Food

The products of the lysophospholipase reaction are free fatty acids and glycerol phosphatides. The lysophospholipase is inactivated by the different manufacturing steps, ie heating, purification and filtration of glucose syrups, under the conditions recommended by the applicant.

3.4. Technological need

The food enzyme would be a processing aid for industrial production of glucose syrup. The conditions of use of the food enzyme in foodstuffs are described by the applicant.

3.5. Dietary exposure

The safety margin is calculated according to the Budget method. The safety margin is calculated using the Budget 8 method. The levels of food consumption are based on physiological consumption a daily consumption of 0.1 l / kg bw of beverages (excl. milk) and 50 g / kg bw of solid food. The calculation of the safety margin is made by considering that 50% of solid foods and 25% of beverages consumed daily by the general population are treated with the enzyme at maximum dose and that the enzymatic activity is retained in full in foodstuffs.

The NOAEL obtained with the sub-chronic oral toxicity study for 90 days in the Rat is 955 mg TOS / kg bw / day. The calculated safety margin, using this NOAEL and the daily intake of lysophospholipase, si then estimated to be of 138405.

3.4 Toxicological data

All toxicity studies were conducted according to OECD international guidelines and in accordance with Good Laboratory Practices.

A dose-dependent increase in serum sodium was observed in males without another change observed on the blood ionogram. The measured data which are in the range of historical laboratory data and other measured data do not lead to suspect impairment of the renal function.

The 90-day sub-chronic oral toxicity study in rats therefore concluded to a NOAEL of 1000 mg of enzyme / kg bw / day, ie 955 mg TOS / kg bw / day, corresponding to the highest tested dose. The in vitro mutagenicity study (Ames test on five histidine dependant strains of *Salmonella typhimurium*) did not reveal any increase in the number of revertants in presence of the food enzyme and therefore no mutagenic effect. The chromosomal aberration test on Chinese hamster pulmonary cells did not provide any evidence of clastogenic or aneugenic effect of the food enzyme. These two tests indicate that the food enzyme is not genotoxic.

3.7.Allergenicity

Singh et al. (2010) showed the allergenic character of two lysophospholipases of a strain of *Aspergillus fumigatus*, PBL1 and PBL3. The sequence comparison of *Aspergillus nishimurae* lysophospholipase with the sequences of these two lysophospholipases showed an identity of 88.14% with PBL 3 and 66.43% with PBL1. These homologies suggest a respiratory allergenic potential of lysophospholipase, subject of the application.

Oral consumption of *Aspergillus nishimurae* lysophospholipase via treated foodstuffs is not a risky route for this allergenic potential. But on enzyme production facility and in plants where the enzyme is used, prevention should be enforced against sensitization by inhalation and by dermal contact with aerosols or particles of this food enzyme, particularly for the powder form of the enzyme. Wearing a mask and protective clothing should prevent the sensitization of operators as already foreseen by the applicant.

3.8 Conclusion of the Committee

In view of the provided results provided and under the intended conditions of use, as described by the applicant in the dossier, the Expert Committee on Biotechnology did not identify any health hazard to the consumer with regard to the use of this lysophospholipase derived from *Trichoderma reesei* (RF7206, RH31920 or CBS125079) carrying a gene from *Aspergillus nishimurae* when used in the manufacturing of glucose syrup.

In accordance with the petitioner's indications, the Expert Committee on Biotechnology recommends respiratory and dermal protection measures for operators in order to prevent sensitization to lysophospholipase.

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

In view of the provided results and under the intended conditions of use, as described by the applicant in the dossier, the National Agency for Food Safety, Environmental and Occupational Health and Safety (ANSES) did not identify any health risk for the consumer in relation to the use of lysophospholipase derived from *Trichoderma reesei* (RF7206, RH31920 or CBS125079) carrying a gene from *Aspergillus nishimurae* when used in the manufacturing of glucose syrup.

Therefore, ANSES gives a favorable opinion to this request.

In accordance with the petitioner's indications, the National Agency for Food Safety, Environmental and Occupational Health and Safety recommends respiratory and dermal protection measures for operators in order to prevent sensitization to lysophospholipase.