



Katherine Vega, Ph.D.
DSM Food Specialties
P.O. Box 1
2600 MA Delft
THE NETHERLANDS

Re: GRAS Notice No. GRN 000857

Dear Dr. Vega:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000857. We received DSM Food Specialties (DSM)'s GRAS notice on April 16, 2019 and filed it on June 28, 2019. We received an amendment containing additional safety information on December 12, 2019.

The subject of the notice is phospholipase A₁ enzyme preparation produced by *Aspergillus niger* genetically engineered to overexpress phospholipase A₁ (phospholipase A₁ enzyme preparation) for use as an enzyme in the degumming of edible vegetable oils, algal oils, fish oils, and animal fats at up to 1.38 mg Total Organic Solids (TOS)/kg oil or fat. The notice informs us of DSM's view that these uses of phospholipase A₁ enzyme preparation are GRAS through scientific procedures.

Commercial enzyme preparations that are used in food processing typically contain an enzyme component that catalyzes the chemical reaction as well as substances used as stabilizers, preservatives, or diluents. Enzyme preparations may also contain components derived from the production organism and from the manufacturing process, e.g., constituents of the fermentation media or the residues of processing aids. DSM's notice provides information about the components in the phospholipase A₁ enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, phospholipase A₁ is identified by the Enzyme Commission Number 3.1.1.32.¹ DSM states that the phospholipase A₁ is 298 amino acids in length with a corresponding molecular weight of 32 kDa.²

DSM describes the construction of the non-pathogenic and non-toxigenic *A. niger* PLN production strain. Multiple expression cassettes, each carrying a gene encoding for phospholipase A₁ from *A. niger plnA*, were inserted by targeted integration into

¹ <https://www.qmul.ac.uk/sbcs/iubmb/enzyme/EC3/1/1/32.html>

² This molecular weight corresponds to the amino acid sequence without the signal sequence.

recipient strain *A. niger* DS 38556³ that was derived from *A. niger* DS 03043. DSM states that the integrations were confirmed by Southern blotting and hybridization. DSM also states that the stability of the introduced DNA has been confirmed by 'end of fermentation' PCR. DSM states that the final production strain does not contain any functional or transferable antibiotic resistance genes.

DSM states that phospholipase A₁ enzyme preparation is manufactured by submerged fed-batch fermentation of a pure culture of the production strain. DSM states that fermentation is carried out under controlled conditions and that the enzyme is secreted into the fermentation media. After fermentation, sodium benzoate is added to the fermentation media at controlled pH and temperature to kill the *A. niger* cells. The enzyme is then recovered from the fermentation media by filtration and concentrated by ultrafiltration. The liquid concentrate is standardized to a liquid enzyme preparation with glycerol. DSM states that the entire process is performed in accordance with current good manufacturing practices. DSM also states that no allergens from the fermentation medium are expected in the final food.⁴

DSM has established food grade specification and states that the phospholipase A₁ enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 11th edition, 2018), and to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing established by the FAO/WHO Joint Expert Committee on Food Additives (JECFA, 2006). DSM provides analytical data from analyses of three batches of phospholipase A₁ enzyme concentrate to demonstrate that the manufacturing specifications have been met, including the absence of the production strain.

DSM intends to use phospholipase A₁ enzyme preparation in the degumming of edible vegetable oils, algal oils, fish oils, and animal fats at 0.35-1.38 mg TOS/kg oil or fat to catalyze the hydrolysis of phospholipids present in these oils and fats. DSM notes that the phospholipase A₁ enzyme preparation will be removed during the refining process. However, in estimating dietary exposure, DSM assumes that all the phospholipase A₁ enzyme preparation will remain in the final food. DSM estimated mean dietary exposure of phospholipase A₁ enzyme preparation from the intended uses to be 0.31-1.22 µg

DSM relies on published information that discusses the safety of microbial enzyme preparations used in food processing, including the safety of the production organism, and the safety of phospholipases. DSM discusses unpublished toxicological studies using a different enzyme produced from a related *A. niger* production strain. FDA notes that these studies only serve to corroborate the safety of the *A. niger* production strain and not the phospholipase A₁ produced by it. In discussing safety of phospholipases, DSM

³ The notifier states that parental strain for this recipient strain is *A. niger* NRRL 3122, which was obtained from the Culture Collection Unit of the Northern Utilization Research and Development Division, US Department of Agriculture.

⁴ DSM states that the intended use of phospholipase A₁ precludes presence of any protein from the process in the finished food.

⁵ DSM estimated dietary exposure to phospholipase A₁ enzyme preparation based on mean consumption of 53 g of oils and solid fats per person per day (USDA, 2014).

states that phospholipase A₁, like other hydrolases used in food processing, represent substances normally consumed by humans.

DSM discusses publicly available literature as well as the conclusions of several organizations and working groups about the low risk of allergenicity posed by enzymes to address potential allergenicity due to phospholipase A₁. Further, based on bioinformatic analyses, DSM reports that the phospholipase A₁ does not share any biologically meaningful sequence homology or sequence identity to potential oral allergens. Based on the totality of the information available, DSM concludes that it is unlikely that oral consumption of phospholipase A₁ enzyme under the intended conditions of use will result in allergic responses.

Based on the data and information summarized above, DSM concludes that phospholipase A₁ enzyme preparation is GRAS for its intended use.

Section 301(ll) of the FD&C Act

Section 301(ll) of the FD&C Act prohibits the introduction or delivery for introduction into interstate commerce of any food that contains a drug approved under section 505 of the FD&C Act, a biological product licensed under section 351 of the Public Health Service Act, or a drug or a biological product for which substantial clinical investigations have been instituted and their existence made public, unless one of the exemptions in section 301(ll)(1)-(4) applies. In our evaluation of DSM's notice concluding that phospholipase A₁ enzyme preparation is GRAS under its intended conditions of use, we did not consider whether section 301(ll) or any of its exemptions apply to foods containing phospholipase A₁ enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing phospholipase A₁ enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that DSM provided, as well as other information available to FDA, we have no questions at this time regarding DSM's conclusion that phospholipase A₁ enzyme preparation produced by *Aspergillus niger* genetically engineered to overexpress phospholipase A₁ is GRAS under its intended conditions of use. This letter is not an affirmation that phospholipase A₁ enzyme preparation produced by *Aspergillus niger* genetically engineered to overexpress phospholipase A₁ is GRAS under 21 CFR 170.35. Unless noted above, our review did not address other provisions of the FD&C Act. Food ingredient manufacturers and food producers are responsible for ensuring that marketed products are safe and compliant with all applicable legal and regulatory requirements.

In accordance with 21 CFR 170.275(b)(2), the text of this letter responding to GRN 000857 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J.
Carlson -S

Digitally signed by Susan
J. Carlson -S
Date: 2019.12.20 14:23:20
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Susan Carlson, Ph.D.
Director
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition