Janet Oesterling
Novozymes North America Inc.
77 Perrys Chapel Church Road, Box 576
Franklinton, NC 27525

Re: GRAS Notice No. GRN 000811

Dear Ms. Oesterling:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000811. We received Novozymes North America Inc. (Novozymes)'s GRAS notice on August 27, 2018, and filed it on September 25, 2018. We received amendments containing additional safety information on April 3, 2019, and April 11, 2019.

The subject of the notice is phospholipase A_1 enzyme preparation produced by *Aspergillus oryzae* expressing a gene encoding phospholipase A_1 from *Valsaria rubricosa* (phospholipase A_1 enzyme preparation) for use as an enzyme at up to 5.8 mg Total Organic Solids (TOS)/kg of flour during the production of baked goods. The notice informs us of Novozymes' view that these uses of phospholipase A_1 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. Novozymes' notice provides information about the components in the phospholipase A_1 enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, phospholipase A_1 is identified by the Enzyme Commission Number 3.1.1.32.[1] Novozymes states that the phospholipase A_1 is 308 amino acids in length with a corresponding molecular weight of 31 kDa.

Novozymes states that the *A. oryzae* production strain AT969 is non-pathogenic and non-toxigenic. AT969 was derived from an *A. oryzae* recipient strain AT604.[2]

[1] https://www.qmul.ac.uk/sbcs/iubmb/enzyme/EC3/1/1/32.html
[2] The notifier states that the recipient strain was derived from Jal731, and its parental strain is a natural isolate of *A. oryzae* A1560. The recipient strain was modified to inactivate genes encoding several amylases and proteases, impair kojic acid production; the aflatoxin gene cluster and a region including a gene involved in cyclopiazonic acid biosynthesis were also deleted.

Novozymes describes the construction of the *A. oryzae* production strain by targeted integration of an expression cassette carrying a gene encoding a phospholipase A_1 from *V. rubricosa*, promoter and terminator elements from *A. niger*, and two selectable markers. Novozymes states that the modification was confirmed by Southern blot, PCR and DNA sequencing. Novozymes also states that the stability of the introduced DNA has been confirmed by Southern blot analysis. Novozymes states that the final production strain does not contain any functional or transferable antibiotic resistance genes.

Novozymes states that phospholipase A_1 enzyme preparation is manufactured by submerged fed-batch fermentation of a pure culture of the production strain. Novozymes states that fermentation is carried out under controlled conditions and that the enzyme is secreted into the fermentation media. After fermentation, flocculants are added to the media containing the desired enzyme, at controlled pH and temperature, to initiate enzyme separation. The enzyme is then recovered from the fermentation media by filtration or centrifugation and concentrated by filtration or evaporation. The liquid concentrate is formulated to a liquid enzyme preparation with sucrose, sorbitol or glycerol, and preserved with potassium sorbate and sodium benzoate, or spray dried to a granulate enzyme preparation with wheat flour. Novozymes states that the entire process is performed in accordance with current good manufacturing practices. Novozymes also states that the final phospholipase A_1 enzyme preparation does not contain any major food allergens from the fermentation media.

Novozymes has established food grade specification and states that the phospholipase A_1 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). Novozymes provides analytical data from analyses of three batches of phospholipase A_1 enzyme concentrate to demonstrate that the manufacturing specifications have been met, including the absence of the production strain.

Novozymes intends to use phospholipase A_1 enzyme preparation at up to 5.8 mg TOS/kg flour to catalyze the hydrolysis of sn-1 ester bond of diacylphospholipids present. Novozymes notes that the phospholipase A_1 enzyme preparation will be deactivated or removed during the baking process. However, in estimating dietary exposure, Novozymes assumes that all the phospholipase A_1 enzyme preparation will remain in the final food. Novozymes estimated dietary exposure of phospholipase A_1 enzyme preparation from baked goods to be 0.052 mg TOS/kg body weight per day (mg TOS/kg bw/d).[3]

Novozymes relies on published information that discusses the safety of microbial enzyme preparations used in food processing, including the safety of the *A. oryzae*

[3] Novozymes uses the Budget method to calculate estimated dietary exposure to phospholipase A_1 enzyme preparation based on consumption of a maximum of 25 g of solid foods per person per day. Novozymes assumes that 50% of all solid foods (i.e., 12.5 g) will be baked goods. Novozymes also assumed that 1 kg of flour will result in 1.4 kg of bread.

production organism, and the safety of phospholipases. Novozymes discusses unpublished toxicological studies using a different enzyme produced from a related *A. oryzae* production strain. FDA notes that these studies only serve to corroborate the safety of the *A. oryzae* production strain and not the phospholipase A_1 produced by it. In discussing safety of phospholipases, Novozymes states that phospholipase A_1, like other hydrolases used in food processing, breaks down substrates into smaller units that are readily metabolized by the human body.

Novozymes 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 A_1. Further, based on bioinformatic analyses, Novozymes reports that the phospholipase A_1 does not share any biologically meaningful sequence homology or sequence identity to potential oral allergens.[4] Based on the totality of the information available, Novozymes concludes that it is unlikely that oral consumption of phospholipase A_1 enzyme will result in allergenic responses.

Based on the data and information summarized above, Novozymes concludes that phospholipase A_1 enzyme preparation is GRAS for its intended use.

Standards of Identity

In the notice, Novozymes states its intention to use phospholipase A_1 enzyme preparation in several food categories, including foods for which standards of identity exist, located in Title 21 of the Code of Federal Regulations. We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity.

Allergen Labeling

The Federal Food, Drug, and Cosmetic Act (FD&C Act) requires that the label of a food that is or contains an ingredient that contains a "major food allergen" declare the allergen's presence (section 403(w)). The FD&C Act defines a "major food allergen" as one of eight foods or food groups (i.e., milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, and soybeans) or a food ingredient that contains protein derived from one of those foods. The phospholipase A_1 enzyme preparation granulate formulation requires labeling under the FD&C Act because it contains protein derived from wheat.

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

[4] Novozymes identified sequence homology to the fungal allergen, Sch c 1, during bioinformatic searches. Novozymes concluded that this is not considered a food allergen. Further, a literature search by the notifier did not reveal any evidence that phospholipase A_1 can trigger oral sensitization.

have been instituted and their existence made public, unless one of the exemptions in section 301(ll)(1)-(4) applies. In our evaluation of Novozymes' notice concluding that phospholipase A_1 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 A_1 enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing phospholipase A_1 enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Novozymes provided, as well as other information available to FDA, we have no questions at this time regarding Novozymes' conclusion that phospholipase A_1 enzyme preparation produced by *A. oryzae* expressing a gene encoding phospholipase A_1 from *V. rubricosa* is GRAS under its intended conditions of use. This letter is not an affirmation that phospholipase A_1 enzyme preparation produced by *A. oryzae* expressing a gene encoding phospholipase A_1 from *V. rubricosa* 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 000811 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2019.07.19 16:38:30 -04'00'

Susan Carlson, Ph.D.
Director
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition