Janet Oesterling
Novozymes NA
77 Perry Chapel Church Rd
Franklinton NC, 27525

Re: GRAS Notice No. GRN 000975

Dear Ms. Oesterling:

This letter corrects our letter dated July 8, 2021, sent in response to GRN 000975. The purpose of this revised letter is to note an amendment to the notice and correct the information reported in the "Conclusions" section of our July 8, 2021 letter.

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000975. We received Novozymes NA's (Novozymes)'s GRAS notice on October 7, 2020 and filed it on January 29, 2021. Novozymes submitted an amendment to the notice on May 19, 2021 that clarified information on the genetic construct and the specifications.

The subject of the notice is maltogenic alpha-amylase enzyme preparation produced by *Bacillus licheniformis* carrying the gene coding for maltogenic alpha-amylase from *Geobacillus stearothermophilus* (maltogenic alpha-amylase enzyme preparation) for use as an enzyme at up to 25 mg Total Organic Solids (TOS)/kg starch-derived dry matter during processing of starch, cereals, and cereal-based beverages, brewing and baking. The notice informs us of Novozymes' view that this use of maltogenic alpha-amylase enzyme preparation is 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 maltogenic alpha-amylase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, maltogenic alpha-amylase is identified by the Chemical Abstracts Service number 160611-47-2 and the Enzyme Commission Number 3.2.1.133.[1] Novozymes states it determined the nucleotide and amino acid

[1] https://www.qmul.ac.uk/sbcs/iubmb/enzyme/EC3/2/1/133.html

sequence, and that the molecular weight of the maltogenic alpha-amylase is 75 kDa.

Novozymes states that the *B. licheniformis* production organism is non-pathogenic and non-toxigenic. Novozymes states that the recipient strain, HyGe735, used in the construction of the production strain, HyGe767n2, was modified to improve product purity and stability.[2] The production strain was constructed by targeted homologous integration of an expression cassette[3] into two loci-specific sites in the genome of the recipient strain. Novozymes states it confirmed the insertion of the expression cassette by whole genome sequencing. Novozymes evaluated the stability of the integration by the large-scale fermentation and production of maltogenic alpha-amylase. Novozymes also verified the absence of functional antibiotic resistance genes in the final production strain genome by sequence analysis.

Novozymes states that the maltogenic alpha-amylase enzyme preparation is manufactured by submerged fermentation of a pure culture of the *B. licheniformis* production strain under controlled conditions, and that the enzyme is secreted into the fermentation broth. Novozymes states that the enzyme is recovered from the microbial biomass by filtration or centrifugation, followed by concentration. After filtration to remove the production organism, the resulting enzyme concentrate is preserved and standardized with sodium chloride to a liquid enzyme concentrate; it is then concentrated, spray- dried and formulated with wheat flour to a maltogenic alpha-amylase enzyme preparation. Novozymes states that the entire process is performed in accordance with current good manufacturing practices using food grade raw materials. Novozymes also states that the fermentation medium used in the manufacturing of maltogenic alpha-amylase enzyme preparation does not contain any major food allergens.

Novozymes has established food-grade specifications and states that the maltogenic alpha-amylase enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 12th edition, 2020), 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 data from analyses of three batches of maltogenic alpha-amylase enzyme to demonstrate that the manufacturing acceptance criteria have been met and that the production organism is absent from the final enzyme preparation.

Novozymes intends to use maltogenic alpha-amylase enzyme preparation at a maximum use level of 25 mg TOS/kg starch-derived dry matter to release alpha-maltose from non-reducing ends of starch. Novozymes notes that the maltogenic alpha-amylase enzyme preparation will be denatured or removed during starch processing. Novozymes estimates a maximum dietary exposure to maltogenic alpha-amylase enzyme preparation to be 0.16 mg TOS/kg body weight per day (mg TOS/kg bw/d) from all the

[2] Modifications include inactivation of genes encoding several proteases, deletion of a gene essential for sporulation and additional genes encoding unwanted proteins in the culture supernatant.
[3] The expression cassette contained the maltogenic alpha-amylase gene *amyM* from *G. stearothermophilus*, engineered promoter containing sequences from *B. amyloliquefaciens* and *B. thuringiensis*, and an engineered transcriptional terminator from *B. clausii* and *B. licheniformis*.

intended uses, and with the assumption that all of it will remain in the final food.[4]

Novozymes relies on published information that discusses the safety of the *B. licheniformis* production organism and the safety of microbial enzyme preparations used in food processing. Novozymes discusses unpublished toxicological studies using a maltogenic alpha-amylase produced by a related *B. licheniformis* production strain. In discussing safety of maltogenic alpha-amylases, Novozymes also states that the notified maltogenic alpha-amylase, like other amylases used in food processing, has a history of use and is ubiquitous to microorganisms, plants, and animals. Additionally, Novozymes found no new studies relevant to the safety of maltogenic alpha-amylase based on literature search performed for the period of 2017-2020. Novozymes corroborates safety of the production strain, HyGe767n2, by discussing unpublished toxicological studies of alpha-amylases produced by genetically engineered *B. licheniformis* predecessor strains to the notified maltogenic-alpha amylase enzyme preparation.

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 maltogenic alpha-amylase. Based on bioinformatic analyses using FARRP allergen protein database, Novozymes reports that *Geobacillus stearothermophilus* maltogenic alpha-amylase shares >35% sequence identity with one mosquito (Aed a 4) and three fungal proteins (Asp o 21, Asp f 13 and Sch c 1) across a window of 80 amino acids. Novozymes states that none of these proteins are registered as food allergens. [5]Novozymes further states that there is no published evidence that Asp f 13, Sch c 1 or Aed a 4, trigger oral sensitization; published reports of potential food allergy to ingested Asp o 21 alpha-amylase were mostly linked to occupational sensitization suggesting that allergic reactions from typical oral exposure is rare. Based on the totality of the information available, Novozymes concludes that it is unlikely that oral consumption of maltogenic alpha-amylase enzyme from the intended use will result in allergenic responses.

Based on the data and information summarized above, Novozymes concludes that maltogenic alpha-amylase enzyme preparation is GRAS for its intended use.

Section 301(ll) of the Federal Food, Drug, and Cosmetic Act (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 Novozymes' notice concluding that maltogenic alpha-amylase 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

[4] Novozymes uses the Budget method to estimate dietary exposure to maltogenic alpha-amylase enzyme preparation based on a maximum of 25% of starch-derived dry matter in processed foods, and consumption of a maximum of 12.5 g of solid foods and 25 mL of beverages per kg body weight per day.
[5]http://www.allergen.org/

containing maltogenic alpha-amylase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing maltogenic alpha-amylase enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

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 bears or contains a "major food allergen" declare the presence of the allergen (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 maltogenic alpha-amylase enzyme preparation requires labeling under the FD&C Act because it is formulated with wheat flour.

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 maltogenic alpha-amylase enzyme preparation produced by *Bacillus licheniformis* carrying the gene coding for maltogenic alpha-amylase from *Geobacillus stearothermophilus* is GRAS under its intended conditions of use. This letter is not an affirmation that maltogenic alpha-amylase enzyme preparation produced by *Bacillus* licheniformis carrying the gene coding for maltogenic alpha-amylase from *Geobacillus stearothermophilus* 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 000975 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2021.08.05 11:26:36 -04'00'

Susan Carlson, Ph.D.
Director
Division of Food Ingredients
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and Applied Nutrition