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

Re: GRAS Notice No. GRN 000751

Dear Ms. Oesterling:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000751. We received Novozymes North America Inc. (Novozymes)'s GRAS notice on December 19, 2017, and filed it on February 6, 2018. We received an amendment containing clarification on the construction of the production strain on July 18, 2018.

The subject of the notice is maltogenic alpha-amylase enzyme preparation produced by *Bacillus subtilis* carrying a maltogenic alpha-amylase gene from *Bacillus stearothermophilus* (maltogenic alpha-amylase enzyme preparation) for use as an enzyme in starch processing, baking, cereal-based processes, and brewing at levels up to 49.5 mg Total Organic Solids per kg (mg TOS/kg) starch raw material. The notice informs us of Novozymes' view that these uses of alpha-amylase 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 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 Enzyme Commission Number 3.2.1.133. The accepted name for this enzyme is glucan 1,4-alpha-maltohydrolase, and the systematic name is 4-alpha-D-glucan alpha-maltohydrolase. The enzyme is also known as maltogenic alpha-amylase, and 1,4-alpha-D-glucan alpha-maltohydrolase. Maltogenic alpha-amylase catalyzes the hydrolysis of (1-4)-alpha-D glucosidic linkages in starch and related polysaccharides and oligosaccharide substrates to remove successive alpha-maltose residues from the non-reducing ends of the chains. The CAS No. for maltogenic alpha-amylase is 160611-47-2.

Novozymes states that the primary amino acid sequence of the mature maltogenic alpha-amylase enzyme has been determined.

Novozymes describes *B. subtilis* as a non-pathogenic, non-toxigenic, well-characterized production organism with a history of safe use in the food industry. Novozymes states that the *B. subtilis* production strain BRG-1 was derived from the *B. subtilis* parental strain A164 via the recipient strain A164Δ5.[1] Novozymes states that the recipient strain was modified at several chromosomal loci to improve the purity and stability of maltogenic alpha-amylase. The modifications include inactivation of genes that encode for proteases, elimination of the ability to sporulate, and deletion of genes that encode unwanted proteins.

Novozymes describes the construction of *B. subtilis* strain BRG-1 by targeted homologous recombination using a plasmid containing an expression cassette carrying the *amyM* gene encoding the maltogenic alpha-amylase from *B. stearothermophilus*, under the control of a promoter derived from *B. amyloliquefaciens* and *B. thuringiensis* and a transcriptional terminator. Novozymes describes the integration of the plasmid containing the above cassette elements to obtain a single copy of the *amyM* gene. The final production strain was obtained by classical mutagenesis of the recombinant bacterium that was screened for increased maltogenic alpha-amylase activity. Novozymes states that only the expression cassette with elements between the promoter and the terminator are present in the final production strain as confirmed by Southern hybridization, PCR, and DNA sequencing. Novozymes also confirmed the absence of functional antibiotic resistance genes in the final production strain by Southern hybridization.

Novozymes states that maltogenic alpha-amylase enzyme is produced 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 medium. After fermentation, maltogenic alpha-amylase is separated from the microbial biomass and recovered by pH adjustment and addition of appropriate flocculants prior to purification. The resulting liquid containing the maltogenic alpha-amylase enzyme is concentrated and filtered to remove residual production strain. This is the test article used in studies to establish safety. The liquid enzyme concentrate is then stabilized by the addition of sucrose, glycerol, and sodium chloride, and preserved by the addition of potassium sorbate and sodium benzoate. The final maltogenic alpha-amylase enzyme preparation is obtained by spray drying to form granules and formulated with wheat flour.

Novozymes states that the entire process is performed in accordance with current good manufacturing practices. Novozymes also states that the maltogenic alpha-amylase enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 10th edition, 2016), 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

[1] *B. subtilis* strain A164 is identical to strain ATCC 6051a, the wild type strain of *B. subtilis*.

states that the maltogenic alpha-amylase enzyme preparation does not contain any major food allergens from the fermentation medium

Novozymes intends to use maltogenic alpha-amylase enzyme preparation during starch processing, baking, cereal-based processes, and brewing at a maximum level of 49.5 mg TOS/kg starch raw material. Novozymes notes that the maltogenic alpha-amylase enzyme preparation is diluted or removed during processing. To estimate dietary exposure to maltogenic alpha-amylase enzyme preparation, Novozymes assumes that the enzyme preparation will be used at the maximum intended levels, and that all the enzyme preparation will remain in the final food. Novozymes estimates dietary exposure from all uses of maltogenic alpha-amylase enzyme preparation to be 0.32 mg TOS/kg body weight per day (mg TOS/kg bw/d).[2]

Novozymes relies on published information that discusses the safety of microbial enzyme preparations used in food processing, including the safety of the production organism. Additionally, Novozymes summarizes unpublished toxicological studies using the maltogenic alpha-amylase enzyme liquid concentrate to corroborate their conclusion of safety of the intended uses. Novozymes states that the maltogenic alpha-amylase enzyme is not mutagenic based on results from a bacterial reverse mutation assay and an *in vitro* chromosomal aberration assay in cultured human lymphocytes. A 13-week oral toxicity study in rats using the maltogenic alpha-amylase enzyme concentrate did not cause any treatment-related adverse effects up to the highest dose tested (equivalent to 969 mg TOS/kg bw/d). Based on the highest dose tested in the 13-week study and the estimated dietary exposure from the intended uses of the maltogenic alpha-amylase enzyme preparation, Novozymes calculates the margin of exposure to be approximately 3000. FDA notes the margin of exposure is based on unpublished safety studies and corroborates the published information regarding enzyme preparations used in food processing.

Novozymes discusses the potential allergenicity of maltogenic alpha-amylase enzyme. Novozymes states that naturally occurring food enzymes, if present in the final food, are unlikely to have allergenic potential because they are present in low concentrations, have history of safe use, or denatured during food processing, and are susceptible to digestion in the gastrointestinal system. Novozymes further cites the conclusions of several organizations and working groups about the low risk of allergenicity posed by enzymes used as processing aids. Additionally, Novozymes conducted sequence homology searches using the amino acid sequence of the maltogenic alpha-amylase against known allergens stored in the FARRP allergen protein database as well as the World Health Organization and International Union of Immunological Societies Allergen Nomenclature Sub-committee. Whereas a search for 100% identity over 8 contiguous amino acids did not produce any hits to known allergens, an 80-mer sliding window search analysis identified one mosquito (Aed a 4) and two fungal (Asp o 21 and

[2] Novozymes uses the Budget Method to calculate estimated dietary exposure to maltogenic alpha-amylase enzyme preparation. Novozymes assumes consumption of a maximum of 25 g of solid foods, and 100 mL of liquid (other than milk) foods per person per day. Novozymes further assumes that 50% of these solid foods containing 25% of starch, and 25% of the liquid foods containing 13% of starch is processed and will contain the maximum level of maltogenic alpha-amylase enzyme preparation.

Sch c 1) allergens that produced homologies above the threshold of 35%. Based on the evaluation of relevant scientific literature, Novozymes concludes that the identified allergens are not expected to result in significant allergy or sensitization from oral exposure. Novozymes also states that homology searches of the maltogenic alpha-amylase sequence using UNIPROT database did not identify any significant homologies to known toxins. Based on the totality of the information available, Novozymes concludes that it is unlikely that oral consumption of maltogenic alpha-amylase enzyme will result in any allergenic or toxigenic responses.

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

Standards of Identity

In the notice, Novozymes states its intention to use maltogenic alpha-amylase enzyme preparation in 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 "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. Maltogenic alpha-amylase enzyme preparation requires labeling under the FD&C Act because it contains wheat flour.

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 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).

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 the maltogenic alpha-amylase enzyme preparation produced by *B. subtilis* carrying a maltogenic alpha-amylase from *B. stearothermophilus* is GRAS under its intended conditions of use. This letter is not an affirmation that maltogenic alpha-amylase enzyme preparation produced by *B. subtilis* carrying a maltogenic alpha-amylase from *B. 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 000751 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Michael A. Adams -S

Digitally signed by Michael A. Adams -S
Date: 2018.07.31 15:42:53 -04'00'

Dennis M. Keefe, Ph.D.
Director
Office of Food Additive Safety
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