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

Re: GRAS Notice No. GRN 000739

Dear Ms. Oesterling:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000739. We received Novozymes North America Inc. (Novozymes)'s GRAS notice on October 20, 2017, and filed it on December 26, 2017. We received an amendment containing additional safety information on March 19, 2018, April 4, 2018, and April 20, 2018.

The subject of the notice is beta-mannanase enzyme preparation produced by *Aspergillus niger* carrying a beta-mannanase gene from *Talaromyces leycettanus* (beta-mannanase enzyme preparation) for use as an enzyme in the production of instant coffee at levels up to 0.7 mg Total Organic Solids (TOS)/g of green coffee bean extract. The notice informs us of Novozymes' view that this use of beta-mannanase 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 beta-mannanase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, beta-mannanase is identified by the Enzyme Commission Number 3.2.1.78. The common name for the enzyme is β-mannanase and the systematic name is mannan endo-1,4- β-mannosidase. Beta-mannanase is also known as endo-1,4-β-mannanase; endo-β-1,4-mannanase; β-mannanase B; β-1,4-mannan 4-mannanohydrolase; endo-β-mannanase; β-D-mannanase. The CAS Registry Number for beta-mannanase is 37288-54-3. Beta-mannanase catalyzes the random hydrolysis of mannoglycosidic bonds in mannans, galactomannans, and glucomannans. Novozymes states that it has determined the beta-mannanase to contain 414 amino acids in its primary amino acid sequence, with a

predicted molecular weight 45 kDa. Novozymes also states that the molecular weight of beta-mannanase to be 50 kDa, as confirmed by the SDS-PAGE analysis. Novozymes describes *A. niger* as a non-pathogenic, non-toxigenic, well-characterized production organism with a history of safe use in the food industry. Novozymes also states that the production strain complies with the Organisation for Economic Co-operation and Development criteria for Good Industrial Large Scale Practice microorganisms. Novozymes states that the *A. niger* production strain, designated 272-C3085-10, was derived from the *A. niger* recipient strain C3085.[1] Novozymes states that the recipient strain was generated through modification at specific chromosomal loci to inactivate genes encoding amylases and proteases, and deletion of the fumonisin gene cluster, the oxaloacetate hydrolase gene, and genes encoding unwanted proteins that can be present in the culture supernatant.

Novozymes describes the construction of the production strain by the targeted integration of an expression cassette containing the beta-mannanase gene from *T. leycettanus,* a fragment of the *A. niger* promoter, a transcriptional terminator from *A. niger*, and a selectable marker. The expression cassette was integrated into four specific loci by targeted homologous recombination. Novozymes confirmed the sequence of the inserted expression cassettes and the flanking regions at each of the integration loci in the production strain via Southern blot hybridization, PCR, and DNA sequencing analyses. Novozymes further confirmed by Southern blot hybridization the genetic stability of the introduced DNA sequences. Novozymes further demonstrated that the transformed DNA is stably integrated into the *A. niger* chromosome, is poorly mobilized for genetic transfer, and is mitotically stable. Novozymes also confirmed by sequence analysis that the production strain does not contain antibiotic resistance genes.

Novozymes states that the beta-mannanase enzyme preparation is manufactured by submerged fermentation of a pure culture prepared from a stock culture of the production strain. Novozymes states that fermentation is carried out under controlled conditions and the culture is periodically tested to ensure production strain identity, purity, and enzyme-generating ability. Novozymes states that the enzyme is secreted into the fermentation medium. After fermentation, the enzyme is recovered by a primary separation step following pH adjustment, and the addition of a flocculating agent. The supernatant containing the enzyme is concentrated by ultrafiltration or evaporation. The concentrated enzyme solution is then filtered again to ensure removal of the production organism. After a final concentration step, the liquid enzyme concentrate is stabilized by the addition of sucrose, sorbitol, and sodium chloride. The stabilized enzyme is further formulated with water, and potassium sorbate and sodium benzoate to preserve it. Novozymes states that the entire process is performed in accordance with current good manufacturing practices using raw materials of food grade quality. Novozymes also states that the final enzyme preparation contains no major food allergens from the manufacturing steps.

Novozymes has established food grade specifications and notes that the beta-mannanase enzyme preparation conforms to specifications established for enzyme

[1] *A. niger* strain C3085 is a natural isolate of *A. niger* strain C40.

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 provides analytical data from three representative batches of beta-mannanase enzyme to demonstrate consistency with the specifications.

Novozymes intends to use beta-mannanase enzyme preparation in the production of instant coffee. The beta-mannanase is used to hydrolyze mannans present in green coffee beans during extraction, to help reduce viscosity of the bean extract, and to increase extraction yield. Novozymes states that the maximum use level of beta-mannanase enzyme preparation for this use will be 0.7 mg TOS/g of green coffee bean extract. Novozymes states that beta-mannanase will be used in processing of instant coffee prior to the pasteurization process that would inactivate the enzyme.[2] However, based on the maximum intended use level and the assumption that all the beta-mannanase enzyme preparation will remain in the final food, Novozymes estimates the dietary exposure to beta-mannanase enzyme preparation to be 0.56 mg TOS/kg bodyweight 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 production organism. Additionally, Novozymes summarizes unpublished toxicological studies using the beta-mannanase enzyme liquid concentrate to corroborate the safety of the enzyme preparation for its intended uses. Novozymes states that the beta-mannanase enzyme is not mutagenic based on results from a bacterial reverse mutation assay and on results from an *in vitro* mouse micronucleus assay in cultured human lymphocytes. A 13-week sub-chronic oral toxicity study in rats using the beta-mannanase enzyme concentrate did not cause any treatment-related adverse effects up to the highest dose tested (equivalent to 1151.7 mg TOS/kg bw/d). Based on the highest dose tested in the 13-week study and the estimated dietary exposure from the intended use of the beta-mannanase enzyme preparation, Novozymes calculates a margin of exposure to be 2057. FDA notes the margin of exposure is based on unpublished safety studies, and serves only to corroborate the published information regarding enzyme preparations used in food processing.

Novozymes discusses the potential food allergenicity of beta-mannanase enzyme. Novozymes conducted an 80-amino acid sequence homology search with the beta-mannanase enzyme sequence against known allergens from the FARRP allergen protein database and found no matches with greater than 35% sequence homology to the allergens in the database. Using the same database, Novozymes did not find any sequence identity to potential allergens in contiguous stretches of eight amino acids within the beta-mannanase enzyme sequence. Novozymes further state that beta-

[2] Novozymes states that the beta-mannanase from *T. leycettanus* has a temperature optimum around 82°C at a pH 5.0. Stability studies at a pH 5.0 at 87° C showed complete inactivation of the beta-mannanase after 30 minutes of incubation.

[3] Novozymes uses the Budget Method to calculate estimated dietary exposure to beta-mannanase enzyme preparation from consumption of a maximum of 25 ml of processed beverages/kg bw/d. Novozymes assumed that all processed beverages are liquid coffee prepared using 5 g of instant coffee/150 ml water.

mannanase is not listed as an allergen within the WHO/IUIS Allergen Nomenclature Sub-committee database. Additionally, Novozymes performed a homology search of the beta-mannanase sequence from *T. leycettanus* to known toxins present in the UNIPROT database; Novozymes states that all homology hits were <17%, indicating that homology to a toxin sequence in the database is low and random and thus not a safety concern. Novozymes further discusses the conclusions of several organizations and working groups about the low-risk of allergenicity posed by enzymes due to their low use levels and the extensive processing of enzyme-containing foods during manufacturing. Based on the totality of the information available, Novozymes concludes that it is unlikely that oral consumption of beta-mannanase enzyme at the intended use level will result in allergenic or toxic responses.

Based on the data and information summarized above, Novozymes concludes that beta-mannanase 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 beta-mannanase 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 beta-mannanase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing beta-mannanase 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 beta-mannanase enzyme preparation produced by *A. niger* carrying a beta-mannanase from *T. leycettanus* is GRAS under its intended conditions of use. This letter is not an affirmation that beta-mannanase enzyme preparation produced by *A. niger* carrying a beta-mannanase from *T. leycettanus* 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 000739 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Michael A. Adams -S

Digitally signed by Michael A. Adams -S
Date: 2018.04.25 08:53:34 -04'00'

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