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

Re: GRAS Notice No. GRN 000680

Dear Ms. Oesterling:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000680. We received Novozymes North America, Inc's (Novozymes') notice on November 14, 2016, filed it on January 9, 2017, and designated it as GRN 000680.

The subject of the notice is alpha-L-arabinofuranosidase enzyme preparation produced by *Trichoderma reesei* expressing a gene coding for alpha-L-arabinofuranosidase from *Talaromyces pinophilus* (alpha-L-arabinofuranosidase enzyme preparation) for use as an enzyme at a maximum level of 31.9 mg Total Organic Solids (TOS) per kg corn dry matter in the wet milling process of corn. The notice informs us of Novozymes' view that this use of alpha-L-arabinofuranosidase 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 components derived from the manufacturing process, e.g., constituents of the fermentation media or the residues of processing aids. Novozymes' notice provides information about each of these components in the alpha-L-arabinofuranosidase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, alpha-L-arabinofuranosidase is identified by the Enzyme Commission Number 3.2.1.55. The accepted name for the enzyme is non-reducing end α-L-arabinofuranosidase, and the systematic names is α-L-arabinofuranoside non-reducing end α-L-arabinofuranosidase. Alpha-L-arabinofuranosidase is also known as arabinosidase; α-arabinosidase; α-L-arabinosidase; α-arabinofuranosidase; polysaccharide α-L-arabinofuranosidase; α-L-arabinofuranoside hydrolase; L-arabinosidase; α-L-arabinanase. The CAS Registry Number for α-L-arabinofuranosidase is 9067-74-7. Alpha-L-arabinofuranosidase catalyzes the hydrolysis of terminal non-reducing α-L-arabinofuranoside residues in α-L-arabinosides.

Novozymes states that the *T. reesei* production strain is constructed using the recipient strain *T. reesei* BTR213. *T. reesei* BTR213 was developed from its parent strain RUTC30.[1] Novozymes describes *T. reesei* as a non-pathogenic, non-toxigenic, and well-characterized production organism with a history of safe use in the food industry. Novozymes also states that *T. reesei* is classified as a Biosafety Level 1 microorganism by the ATCC.

Novozymes describes the construction of the production strain from *T. reesei* BTR213 by the targeted integration of an expression cassette carrying the alpha-L-arabinofuranosidase gene (*afuTP*) from *T. pinophilus,* a fragment of the *T. reesei* cellobiohydrolase 1 (*cbh1*) promoter, the transcriptional terminator of *cbh1,* and an acetamidase selectable marker (*amdS*). Novozymes confirmed the sequence of the inserted expression cassette and the flanking regions at the integration locus. Novozymes also confirmed that the introduced DNA is stable during production via Southern blot hybridization, and free of vector backbone, including the functional antibiotic resistance genes, via genome sequence analysis.

Novozymes states that the alpha-L-arabinofuranosidase enzyme preparation is produced by submerged fermentation of a pure culture of the production strain, controlled to ensure production strain identity, purity, and enzyme-generating ability. Novozymes states that each batch of the fermentation process is initiated with a stock culture of the production organism. The enzyme is secreted into the fermentation medium, and it is recovered first by pH adjustment and flocculation as needed, followed by a primary separation step of filtration or centrifugation. The filtrate containing the enzyme is then concentrated via ultrafiltration or evaporation. The resulting liquid concentrate is further filtered to remove any residual production organism followed by a final concentration step. The liquid enzyme concentrate is then standardized with sucrose and preserved with the addition of potassium sorbate and sodium benzoate. 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 fermentation media.

Novozymes has established food grade specifications and notes that the alpha-L-arabinofuranosidase enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 9th edition, 2014), and to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2006). Novozymes provides analytical data from three batches of alpha-L-arabinofuranosidase enzyme preparation to demonstrate consistency with the specifications. Novozymes states that absence of the production microorganism is an established specification for the commercial product.

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[1] *T. reesei* RUTC30 has been deposited in ATCC (ATCC 56765); it was developed from the well characterized wild type strain QM6a. QM6a has been used in the construction of several enzyme production strains used for industrial scale food processing applications.

Novozymes intends to use the alpha-L-arabinofuranosidase enzyme preparation at levels up to 31.9 mg TOS per kg of corn dry matter to increase the yield of starch and gluten meal during the wet milling process of corn. The alpha-L-arabinofuranosidase enzyme cleaves the highly branched arabinoxylans present in the outer cell walls and endosperm of cereal grains such as corn, wheat, barley, rye, and oat. Novozymes states that the alpha-L-arabinofuranosidase enzyme will be inactivated during processing, and is not expected to be functional in the final food. Novozymes estimates dietary exposure to alpha-L-arabinofuranosidase enzyme preparation based on the maximum intended use levels and the assumption that all of the enzyme preparation will remain in the final food, to be 0.146 mg TOS/kg bw/d.

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 alpha-L-arabinofuranosidase enzyme concentrate to corroborate the safety of the enzyme preparation for its intended uses. Novozymes states that the alpha-L-arabinofuranosidase 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 oral toxicity study showed that rats fed with alpha-L-arabinofuranosidase enzyme concentrate did not have any treatment-related adverse effects up to the highest dose tested (equivalent to 1116 mg TOS/kg bw/d). Based on the highest dose tested in the 90-day study and a theoretical maximum estimated dietary intake of 0.146 mg TOS/kg bw/d from the intended use of the alpha-L-arabinofuranosidase enzyme preparation, Novozymes calculates a margin of safety to be 7644.

Novozymes discusses potential food allergenicity of alpha-L-arabinofuranosidase enzyme. Novozymes states that naturally occurring food enzymes, if present in the final food, are susceptible to digestion in the gastro-intestinal system, and are unlikely to have allergenic potential because they are present in low concentrations. Novozymes conducted an 80-amino acid sequence homology search for alpha-L-arabinofuranosidase against known allergens stored in the FARRP allergen protein database at the recommended default settings. Novozymes found no sequence homology over 35% to any known allergens using a window of 80 amino acids. Novozymes also did not find any significant matches of contiguous stretches of eight or more amino acids within the alpha-L-arabinofuranosidase sequence against proteins in the FARRP allergen database. In addition, Novozymes reported no sequence homologies of >20% for the alpha-L-arabinofuranosidase when compared to known toxin sequences present in the UNIPROT database. Novozymes cites 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 alpha-L-arabinofuranosidase enzyme will result in any allergenic responses.

Based on the data and information summarized above, Novozymes concludes that alpha-L-arabinofuranosidase enzyme preparation is GRAS for its intended use in food.

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 the alpha-L-arabinofuranosidase 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 alpha-L-arabinofuranosidase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing alpha-L-arabinofuranosidase 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 alpha-L-arabinofuranosidase enzyme preparation produced by *T. reesei* expressing a gene coding for alpha-L-arabinofuranosidase from *T. pinophilus* is GRAS under its intended conditions of use. This letter is not an affirmation that alpha-L-arabinofuranosidase enzyme preparation produced by *T. reesei* expressing a gene coding for alpha-L-arabinofuranosidase from *T. pinophilus* 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 000680 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Michael A. Adams -S

Digitally signed by Michael A. Adams -S
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Dennis M. Keefe, Ph.D.
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