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

Re: GRAS Notice No. GRN 001030

Dear Ms. Oesterling,

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001030. We received the notice you submitted on behalf of Novozymes North America Inc. (Novozymes) on March 8, 2021 and filed it on January 14, 2022. Novozymes submitted amendments to the notice on October 7, 2022 and February 2, 2022, providing clarification on identity, specifications, and the safety narrative.

The subject of the notice is cellulase enzyme preparation produced by *Aspergillus niger* genetically engineered to express a cellulase gene from *Trichoderma reesei* (cellulase enzyme preparation) for use as an enzyme at a maximum level of 100 mg total organic solids/kg raw material in brewing and processing of other cereal based beverages, fruits and vegetables, starches and grains, and baked goods. The notice informs us of Novozymes' view that this use of cellulase 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 cellulase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, cellulase is identified by the Chemical Abstracts Service number 9012-54-8 and the Enzyme Commission Number 3.2.1.4.[1] Novozymes states that the primary sequence of cellulase is 327 amino acids with a molecular weight of 35 kDa.

Novozymes states that the *A. niger* production organism is non-pathogenic and non-toxigenic. Novozymes states that the recipient strain, used in the construction of the

[1] https://iubmb.qmul.ac.uk/enzyme/EC3/2/1/4.html

production strain *A.niger* C3085-1870-2, was obtained by mutagenesis of a natural isolate, *A. niger* C40. Novozymes states that the production organism was constructed through transformation of the recipient strain with an expression cassette carrying the neutral amylase promoter from *A. niger*, the cellulase coding sequence, a transcriptional terminator, and *amdS* encoding acetamidase as a selective marker. Novozymes states that it confirmed sequence integration by whole genome sequencing. Novozymes evaluated the genetic stability of the production strain by measuring the enzyme activity in three independent batches of the food enzyme. Novozymes states that the expression cassette is integrated into the *A. niger* and is poorly mobilized for genetic transfer. Novozymes also verified the absence of functional antibiotic resistance genes by genome sequencing.

Novozymes states that the cellulase enzyme preparation is manufactured by submerged fermentation of a pure culture of the *A. niger* C3085-1870-2 production strain under controlled conditions. The cellulase enzyme is secreted into the fermentation medium and then recovered by a series of steps including pH adjustment, flocculation, filtration (including germ filtration), centrifugation, concentration, preservation, and stabilization (with sodium chloride and sucrose). Novozymes states that the fermentation medium does not contain any major food allergens. Novozymes states that the entire process is performed using food grade raw materials and in accordance with current good manufacturing practices.

Novozymes has established food grade specifications and states that the cellulase enzyme preparation conforms to the specifications set in the Food Chemicals Codex (FCC, 12th ed., 2020)[2] 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 results from analyses of three non-consecutive batches of cellulase enzyme preparation to demonstrate that the manufacturing acceptance criteria have been met, including the absence of both the production organism and antibiotic activity.

Novozymes intends to use cellulase enzyme preparation to catalyze the hydrolysis of the 1,4-beta-D-glycosidic linkages in cellulose, hemicellulose, lichenin, and cereal beta-D-glucans to break down the cellulose present in plants' cell walls. Novozymes notes that cellulase enzyme will be added during the milling, mixing, or steeping of grains, dough mixing stage of baked goods, mashing step of beermaking, and extraction and clarification processes of juice making. Novozymes states that no enzyme activity is expected to be present in the final food products, since the enzyme is either heat inactivated or removed during processing. Novozymes estimates a maximum dietary exposure to cellulase enzyme preparation to be 5.56 mg TOS/kg body weight per day (mg TOS/kg bw/d) under the assumption that all the cellulase enzyme preparation will remain present in the final food products.[3]

[2] Specifications for enzymes remain the same in the most recent edition of the Food Chemicals Codex (FCC,13th edition, 2022).

[3] Novozymes uses a combination of two methods to estimate exposure to cellulose enzyme: the Budget method for starch/grain processing and sum of 95th percentile estimated dietary intakes of specified food categories, with the

Novozymes relies on published information that discusses the safety of the *A. niger* production organism and the safety of microbial enzyme preparations used in food processing. Novozymes states that the donor organism, *T. reesei* (ATCC 56765), which provided the gene used to produce the cellulase enzyme preparation that is the subject of this notice, was also used in the development of the *T. reesei* strain used in the production of the cellulase enzyme preparation that is the subject of 21 CFR 184.1250. Novozymes provides the results of a literature search that found no indication of toxicity or adverse events associated with cellulase enzyme.

Novozymes discusses publicly available literature that indicates low risk of allergenicity posed by oral consumption of enzymes to address potential allergenicity due to cellulase. Based on bioinformatic analyses, Novozymes reports no matches were found between the amino acid sequences of the cellulase enzyme and the primary sequences of known allergens based on the guidelines developed by Codex Alimentarius Commission (FAO, 2009). Based on the provided information, Novozymes concludes that consumption of the cellulase enzyme preparation is unlikely to present an allergenicity concern.

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

Standards of Identity

In the notice, Novozymes states its intention to use cellulase enzyme preparation in several food categories, including foods for which standards of identity exist, located in Title 21 of the CFR. 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.

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

---

latter based on consumption data outside the US. For comparison, FDA prepared its own estimate of exposure from all food categories using the cited Budget method and Novozyme's assumptions regarding the portion of total food (50% of 25 g/kg bw/d) and beverage (25% of 100 ml/kg bw/d non-milk liquids) that is processed. Assuming maximum levels of 100 mg TOS per kg raw material and the maximum proportions of raw material per kg food described in GRN 001030, the estimated dietary exposure would be approximately 3.8 mg TOS/kg bw/d. Based on the similarity of Novozyme's estimates to those prepared using the Budget method, FDA did not have questions regarding Novozyme's estimates of dietary exposure and resulting GRAS conclusions.

cellulase 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 cellulase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing cellulase 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 cellulase enzyme preparation produced by *A. niger* genetically engineered to express a cellulase gene from *T. reesei* is GRAS under its intended conditions of use. This letter is not an affirmation that cellulase enzyme preparation produced by *A. niger* 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 001030 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2023.02.15 13:46:24 -05'00'

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