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

Re: GRAS Notice No. GRN 001055

Dear Ms. Oesterling,

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001055. We received Novozymes North America Inc.'s (Novozymes) notice on January 17, 2022 and filed it on June 9, 2022. Novozymes submitted amendments to the notice on February 10, 2023 and March 7, 2023 that provided clarifications on the identity, specifications, and dietary exposure.

The subject of the notice is xylanase enzyme preparation produced by *Bacillus licheniformis* expressing a gene encoding xylanase from *Chryseobacterium cucumeris* (xylanase enzyme preparation) for use as an enzyme at up to 68.5 mg total organic solids (TOS)/kg starch in grain processing, brewing, and manufacture of cereal-based beverages. The notice informs us of Novozymes's view that this use of xylanase 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's notice provides information about the components in the xylanase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, xylanase is identified by the Chemical Abstracts Service number 9025-57-4 and the Enzyme Commission Number 3.2.1.8.[1] Novozymes states that the primary sequence of xylanase is 551 amino acids with a calculated molecular weight of 60.8 kDa.

Novozymes states that the *B. licheniformis* production organism is non-pathogenic and non-toxigenic. Novozymes states the recipient strain used in the construction of the production strain, SJ14481, was genetically engineered from a natural isolate of *B. lichenformis* strain DSM 9552 through inactivation of several protease genes, deletion of

[1] https://iubmb.qmul.ac.uk/enzyme/EC3/2/1/8.html

a sporulation gene, and deletion of additional genes encoding unwanted proteins. Novozymes states that the production organism was constructed through transformation with an expression cassette carrying a *B. lichenformis* promoter, the *C. cucumeris* modified xylanase gene, and a transcriptional terminator.[2] Novozymes states that they confirmed sequence integrity by whole genome sequencing. Novozymes confirmed the genetic stability of the production strain by measuring enzyme activity and protein expression. Novozymes also verified the absence of functional resistance genes and transferrable antibiotic resistance genes in the final production strain by genome sequencing.

Novozymes states that the xylanase enzyme preparation is produced by a submerged fed-batch fermentation of a pure culture of the *B. licheniformis* SJ14481 production strain under controlled conditions. The xylanase is secreted into the fermentation medium and then recovered by pretreatment via pH adjustment and flocculation then separated via filtration or centrifugation. This is followed by concentration and multiple filtration steps. The resulting liquid enzyme concentrate is tan to brown and is stabilized with sorbitol and preserved with potassium sorbate and sodium benzoate. Novozymes states that the fermentation medium does not contain any major allergens or components derived from allergenic sources. Novozymes states that the entire process is performed using food grade raw materials and in accordance with good manufacturing practices.

Novozymes has established food grade specifications and states that the xylanase enzyme preparation conforms to the specifications set in the Food Chemicals Codex (FCC, 9th ed., 2014)[3] 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 xylanase enzyme preparation to demonstrate that the manufacturing acceptance criteria have been met, including the absence of the production organism and antibiotic activity.

Novozymes intends to use xylanase enzyme preparation at a maximum use level of 68.5 mg TOS/kg starch in grain processing, brewing, and manufacture of cereal-based beverages. Novozymes states that the xylanase hydrolyses xylosidic linkages in the arabinoxylan backbone resulting in a depolymerization of arabinoxylans into smaller oligosaccharides. Novozymes notes that the xylanase enzyme preparation is inactivated or denatured during processing. Novozymes estimates a maximum dietary exposure to the xylanase enzyme preparation to be 0.34 mg TOS/kg body weight/day from the intended uses, with the assumption that the xylanase remains present in the final food.[4]

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[2] Novozymes states that the production strain is deposited with a known culture collection.

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

[4] Novozymes uses a combination of the Budget method and 2003-2012 NHANES beer 90th percentile estimated dietary intakes. For comparison, FDA prepared its own estimate using the cited Budget method, and the assumptions for the total portion of processed food containing starch (12.5% of 25 g/kg bw/d for solid food and 5% of 100 mL/kg bw/d for beverages). Assuming maximum intended use levels

Novozymes relies on published information that discusses the safety of the *B. licheniformis* production organism, including safe strain lineage and use of the parent strain for production of food ingredients, and the safety of microbial enzyme preparations used in food processing. Novozymes discusses unpublished toxicological studies using *B. licheniformis* to produce other enzymes. Novozymes also discusses unpublished toxicological studies using their article of commerce as test article and concludes that the enzyme preparation is not genotoxic nor showed any toxicologically significant adverse effects at any dose tested in a 90-day repeat dose oral toxicity study in rats. In support of the safety of xylanase enzyme preparation, Novozymes highlights the history of safe use of xylanase in food. Additionally, Novozymes states that a literature search did not identify any information that would contradict a general recognition of safety of xylanase enzyme preparation.

Novozymes discusses publicly available literature to address potential allergenicity due to xylanase. Based on bioinformatic analyses, Novozymes reports no significant matches between the amino acid sequences of the xylanase and the primary sequences of known food allergens based on the guidelines developed by the FAO/WHO in 2001 (Food and Agriculture Organization of the United Nations, January 2001) and the Codex Alimentarius Commission in 2009 (Codex, 2009). Based on the totality of information available, Novozymes concludes that it is unlikely that oral consumption of xylanase enzyme preparation from the intended uses will result in allergic responses.

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

Standards of Identity

In the notice, Novozymes states its intention to use xylanase 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 xylanase enzyme preparation is GRAS under its intended conditions of use, we did not

for grain of 39 mg TOS/kg starch and for beverages/brewing of 68.5 mg TOS/kg starch, the estimated dietary intake would be 0.46 mg TOS/kg bw/d. Based on the similarity of Novozyme's estimate to those prepared using the Budget method, FDA does not have questions regarding Novozyme's estimate of dietary exposure and the resulting GRAS conclusions.

consider whether section 301(ll) or any of its exemptions apply to foods containing xylanase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing xylanase 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 xylanase enzyme preparation produced by *B. licheniformis* expressing a gene encoding xylanase from *C. cucumeris* is GRAS under its intended conditions of use. This letter is not an affirmation that xylanase enzyme preparation produced by *B. licheniformis* expressing a gene encoding xylanase from *C. cucumeris* 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 001055 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2023.04.04 15:16:37 -04'00'

Susan Carlson, Ph.D.
Director
Division of Food Ingredients
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