Janet Oesterling
Novozymes North America
77 Perrys Chapel Church Rd., Box 576
Franklinton, NC 27525

Re: GRAS Notice No. GRN 001120

Dear Ms. Oesterling:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001120. We received Novozymes North America (Novozymes)'s GRAS Notice on December 9, 2022, and filed it on May 24, 2023. Novozymes submitted amendments to the notice on April 4, 2024 and April 23, 2024 containing additional information on enzyme identity, intended use, specifications, and analytical methods.

The subject of the notice is α-galactosidase enzyme preparation produced by *Aspergillus niger* (α -galactosidase enzyme preparation) for use as an enzyme at up to 2.54 g Total Organic Solids (TOS)/kg sugars in legumes in the production of plant-based yogurt and dairy analogues, as well as plant-based meat analogues. The notice informs us of Novozymes' view that this use of α-galactosidase 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 α-galactosidase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, α-galactosidase is identified by the Enzyme Commission Number 3.2.1.22,[1] and the Chemical Abstracts Service Number of 9025-35-8. Novozymes states that the primary amino acid sequence of the α-galactosidase consists of 747 amino acids with a calculated molecular weight of 88 kDa.

Novozymes states that the *A. niger* production organism is a non-pathogenic and non-toxigenic fungus. Novozymes states that the *A. niger* production strain 1633-C4645-3 was produced by integration of an expression cassette carrying an *A. niger* α-

[1] https://iubmb.qmul.ac.uk/enzyme/EC3/2/1/22.html

galactosidase gene, promotor and terminator, and an *Aspergillus nidulans* selectable marker into four loci of the recipient strain C4645. Novozymes states that sequencing was used to confirm the sequence integrity of the inserted expression cassettes and flanking regions. Novozymes confirmed the genetic stability of the production strain by measuring the α-galactosidase enzyme activity and protein expression profile. Novozymes also states that the production strain does not contain any functional antibiotic resistance genes.

Novozymes states that α-galactosidase enzyme preparation is manufactured by controlled fermentation of a pure culture of the *A. niger* production strain. The enzyme is secreted into the fermentation medium. After fermentation, the medium containing the enzyme is separated from the biomass, recovered, and concentrated by evaporation and/or ultrafiltration steps. The resulting α-galactosidase enzyme concentrate is stabilized with sorbitol and glycerol and formulated to a liquid enzyme preparation with water and preserved with potassium sorbate and sodium benzoate. The final α-galactosidase enzyme preparation is a transparent light brownish liquid. Novozymes states that the entire process is performed in accordance with current Good Manufacturing Practices and with food-grade raw materials. Novozymes also states that the α-galactosidase enzyme preparation does not contain any major food allergens.

Novozymes has established food-grade specifications and states that the α-galactosidase enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 13th edition, 2022), 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 α -galactosidase enzyme concentrate to demonstrate that the manufacturing acceptance criteria can be met, including the absence of the production organism in the final product.

Novozymes states α-galactosidase enzyme preparation is intended for use at a maximum level of 2.54 g TOS/kg sugars in legumes in the production of plant-based yogurt and dairy analogues, as well as plant-based meat analogues. α-galactosidase catalyzes the hydrolysis of terminal, non-reducing α-D-galactose residues in α-D-galactosides, including galactooligosaccharides, galactomannans and galactolipids. Novozymes notes that the α-galactosidase enzyme is removed or denatured during food production. Novozymes estimates a maximum dietary exposure to the α-galactosidase enzyme preparation to be 4.66 mg TOS/kg body weight (bw)/day TOS/kg bw/day from the use in food and drinks with the assumption that all of the α-galactosidase enzyme will be active and remain in the final food.[2]

Novozymes relies on published information that discusses the safety of the *A. niger* α-galactosidase, the *A. niger* production organism and the safety of microbial enzyme preparations used in food processing. Novozymes summarizes the results of

[2]Novozymes uses the Budget method to estimate the dietary exposure to α-galactosidase enzyme preparation based on a maximum use levels of 2.54 g TOS/kg sugars in legumes, sugar contents of 7.33 g sugar/100g soybeans and 3.65 g sugar/100g soy milk, respectively, and consumption of 12.5 g of solid foods and 25 mL of beverages per kg body weight per day.

unpublished toxicological studies using the α-galactosidase enzyme concentrate. This includes a reverse mutation assay, an *in vitro* micronucleus assay, and a 13-week oral toxicity study using the α-galactosidase enzyme concentrate. Novozymes states that the α-galactosidase enzyme preparation was not mutagenic, and there were no treatment-related adverse effects up to the highest dose tested (1305 mg TOS/kg bw/d). Novozymes calculates a margin of exposure to be 280 using the NOAEL and the estimated dietary exposure for the intended uses of the α-galactosidase enzyme preparation. FDA notes the margin of exposure is based on unpublished safety studies and is corroborative.

Novozymes discusses publicly available literature, as well as the conclusions of several organizations and working groups, about the low risk of allergenicity posed by enzymes from their intended use, to address potential allergenicity due to α-galactosidase. Based on bioinformatic analyses, using criteria recommended by FAO/WHO (FAO/WHO, 2001; Codex Alimentarius, 2009; JECFA, 2016), Novozymes reports that no sequence homology of *A. niger* α-galactosidase to known allergens that would raise allergenicity concerns were identified. Based on the totality of the information available, Novozymes concludes that it is unlikely that oral consumption of α-galactosidase will result in allergenic responses from its intended uses.

Based on the data and information summarized above, Novozymes concludes that α-galactosidase 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 α-galactosidase 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 α-galactosidase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing α-galactosidase 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 α-galactosidase enzyme preparation is GRAS under its intended conditions of use. This letter is not an affirmation α-galactosidase enzyme preparation 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 001120 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2024.06.05 18:36:52 -04'00'

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