



Lori Gregg
Novozymes North America
77 Perrys Chapel Church Rd., Box 576
Franklinton, NC 27525

Re: GRAS Notice No. GRN 001139

Dear Ms. Gregg:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001139. We received Novozymes North America (Novozymes)'s GRAS on April 3, 2023, and filed it on August 18, 2023. Novozymes submitted amendments to the notice on May 14, 2024 and June 13, 2024 containing additional information on enzyme identity, manufacturing, specifications, and analytical methods.

The subject of the notice is transglutaminase enzyme preparation produced by *Bacillus licheniformis* carrying a gene encoding transglutaminase from *Streptomyces mobaraensis* (transglutaminase enzyme preparation) for use as an enzyme at up to 1.2 g Total Organic Solids (TOS)/kg protein in the production of dairy foods (milk, fermented milk, yogurt, cheese), plant-based dairy analogues, plant-based egg analogues, plant-based meat analogues, tofu, processed fish (non-Siluriformes only), seafood and plant-based seafood analogues, baked goods, cereals and grain-based products, in brewing, and other cereal-based or plant-based beverages. The notice informs us of Novozymes' view that this use of transglutaminase 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 transglutaminase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, transglutaminase is identified by the Enzyme Commission Number 2.3.2.13,¹ and the Chemical Abstracts Service Number 80146-85-6. Novozymes states that the primary amino acid sequence of the

¹ <https://iubmb.qmul.ac.uk/enzyme/EC2/3/2/1322.html>

transglutaminase consists of 331 amino acids with a calculated molecular weight of 38 kDa.

Novozymes states that the *B. licheniformis* production organism is a non-pathogenic and non-toxicogenic bacterium. Novozymes states that the *B. licheniformis* production strain JA4767 was produced by integration of an expression cassette carrying a *S. mobaraensis* transglutaminase gene, and hybrid *Bacillus* promoter and terminator, into three loci of the recipient strain SJ13672. Novozymes states that PCR and DNA sequencing was used to confirm the sequence integrity of the inserted expression cassettes. Novozymes also states that based on the results of genome sequencing, the production strain does not contain any functional antibiotic resistance genes.

Novozymes states that transglutaminase enzyme preparation is manufactured by controlled fermentation of a pure culture of the *B. licheniformis* 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 transglutaminase enzyme concentrate is stabilized with glycerol and formulated to a liquid enzyme preparation with water. 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 transglutaminase enzyme preparation does not contain any major food allergens.

Novozymes has established food-grade specifications including a limit for lead (< 0.5 mg/kg) and states that the transglutaminase 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 transglutaminase enzyme concentrate to demonstrate that the manufacturing acceptance criteria have been met, including the absence of the production organism in the final product.

Novozymes states transglutaminase enzyme preparation is intended for use at up to 1.2 g TOS/kg protein in food and beverage production. Transglutaminase catalyzes the formation of an ϵ -(γ -glutamyl) lysine bond between the carboxamide group of protein glutamine residues with the ϵ - amino group of protein lysine residues. Novozymes notes that the transglutaminase enzyme is denatured, inactivated due to pH changes, or removed during food production. However, Novozymes estimates a maximum dietary exposure to the transglutaminase enzyme preparation to be 2.9 mg TOS/kg body weight (bw)/day (TOS/kg bw/day) from the intended uses, with the assumption that the transglutaminase enzyme preparation remains present in the final food.²

² Novozymes uses the Budget method to estimate the dietary exposure to the transglutaminase enzyme preparation based on the consumption of 12.5 g solid food and 25mL non-milk beverages per kg body weight per day (worst case scenario) containing the transglutaminase enzyme preparation at the recommended use level.

Novozymes relies on published information that discusses the safety of the transglutaminase, the *B. licheniformis* production organism and the safety of microbial enzyme preparations used in food processing. Novozymes states that a literature search did not identify any information that would contradict a general recognition of safety of the transglutaminase enzyme preparation. Additionally, Novozymes summarizes the results of unpublished toxicological studies using the transglutaminase enzyme concentrate. This includes a bacterial reverse mutation assay, an *in vitro* micronucleus assay, and a 13-week oral toxicity study in rats using the transglutaminase enzyme concentrate. Novozymes states that the transglutaminase enzyme preparation was not mutagenic, and there were no treatment-related adverse effects up to the highest dose tested (372 mg TOS/kg bw/d). Novozymes calculates a margin of exposure to be 128 using the No Observed Adverse Effect Level (NOAEL) and the estimated dietary exposure for the intended uses of the transglutaminase 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 transglutaminase. 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 transglutaminase to known allergens that would raise allergenicity concerns were identified. Novozymes also discusses publications that associate transglutaminase consumption with celiac disease. However, Novozymes notes that the transglutaminase enzyme is inactivated in the final food product and would be digested in the gastrointestinal tract, thus limiting dietary exposure to the transglutaminase enzyme. Based on the totality of the information available, Novozymes concludes that it is unlikely that oral consumption of transglutaminase will result in allergenic or adverse immune responses from its intended uses.

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

Section 301(l) of the Federal Food, Drug, and Cosmetic Act (FD&C Act)

Section 301(l) 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(l)(1)-(4) applies. In our evaluation of Novozymes' notice concluding that transglutaminase enzyme preparation is GRAS under its intended conditions of use, we did not consider whether section 301(l) or any of its exemptions apply to foods containing transglutaminase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing transglutaminase enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(l).

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 transglutaminase enzyme preparation is GRAS under its intended conditions of use. This letter is not an affirmation that transglutaminase 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 001139 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

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