Annie Han
Danisco US Inc. (A Wholly Owned-Subsidiary of International Flavors & Fragrances)
925 Page Mill Road
Palo Alto, CA 94304

Re: GRAS Notice No. GRN 001048

Dear Ms. Han:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001048. We received Danisco US Inc.'s[1] (Danisco) notice on December 30, 2021 and filed it on March 11, 2022. Danisco submitted amendments to the notice May 26, 2023[2] and July 6, 2023 that provided additional information on the production organism, enzyme identity, manufacturing, and analytical methods.

The subject of the notice is aminopeptidase Y enzyme preparation produced by *Trichoderma reesei* expressing a gene encoding an aminopeptidase Y from *Aspergillus clavatus* (aminopeptidase Y enzyme preparation) for use as an enzyme at up to 2.1 g total organic solids (TOS)/kg protein in protein processing, at up to 4.3 g TOS/kg yeast in yeast processing, and at up to 7 g TOS/kg protein in flavoring production. The notice informs us of Dansico's view that this use of aminopeptidase Y 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. Danisco's notice provides information about the components in the aminopeptidase Y enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, aminopeptidase Y is identified by the Enzyme Commission Number 3.4.11.15;[3] the CAS Number for aminopeptidase Y is 114796-97-3. Danisco provides the primary amino acid sequence of the aminopeptidase

[1] Danisco US Inc. is a wholly owned subsidiary of International Flavors & Fragrances Inc.

[2] The amendment dated May 26, 2023 designated the strain name provided in the GRAS notice as confidential and provided alternate strain names. The alternate strain name designation in Danisco's culture collection is GICC03501 and the deposit designation for Westerdijk Fungal Biodiversity Institute (The Netherlands) is CBS 145613.

[3] https://iubmb.qmul.ac.uk/enzyme/EC3/4/11/15.html

Y consisting of 455 amino acids and states it has a calculated molecular weight of 50 kDa.

Danisco states that the *T. reesei* production organism is a non-pathogenic and non-toxigenic fungus with a history of safe use in food production. Danisco states that the *T. reesei* production strain CBS 145613 was constructed from the host strain by targeted integration of an expression cassette carrying a codon optimized gene encoding aminopeptidase from *Aspergillus clavatus* under control of a promoter and a terminator from *T. reesei* and selectable markers. Danisco states that whole genome sequencing was used to confirm the sequence integrity of the inserted sequences and PCR was used to confirm genetic stability. Danisco states that the final production strain does not contain any functional or transferable antibiotic resistance genes.

Danisco states that aminopeptidase Y enzyme preparation is manufactured by submerged fermentation of a pure culture of the *T. reesei* production strain under controlled conditions. The enzyme is secreted into the fermentation medium and then recovered by centrifugation or filtration, before being concentrated by ultrafiltration. The resulting aminopeptidase Y enzyme concentrate is formulated to a liquid preparation with glycerol, sodium chloride, sodium benzoate, potassium sorbate, calcium chloride, and water. Danisco states that the entire process is performed in accordance with current Good Manufacturing Practices and with raw materials that are food-grade. Danisco also states that the fermentation medium used in the manufacturing of aminopeptidase Y enzyme preparation contains glucose, which may be derived from wheat, and does not contain any other major food allergens.

Danisco has established food-grade specifications and states that the aminopeptidase Y 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). Danisco provides data from analyses of three batches of aminopeptidase Y enzyme preparation to demonstrate that the manufacturing acceptance criteria can be met, including the absence of the production organism.

Danisco states that the aminopeptidase Y enzyme preparation is intended for use at a maximum level of 2.1 g TOS/kg protein in protein processing, 4.3 g TOS/kg yeast in yeast processing, and 7 g TOS/kg protein in flavoring production to catalyze the cleavage of the N-terminal peptide bond of the proteins and peptides to release an N-terminal residue. Danisco notes that the aminopeptidase Y enzyme will be inactivated or removed during food production. Danisco estimates a maximum dietary exposure to aminopeptidase Y enzyme preparation to be 2.26 TOS/kg bw/day from these uses with the assumption that the added aminopeptidase Y enzyme preparation will remain in the final food.[4]

[4] Danisco uses the Budget Method to estimate the dietary exposure to aminopeptidase Y enzyme preparation based on the consumption of 25 g of solid food per kg bw/d. Danisco assumes that 50% of the solid foods (12.5 g/kg bw/d) will be used for protein processing (worst case scenario) and contain the aminopeptidase Y enzyme preparation at the maximum use level.

Danisco relies on published information that discusses the safety of the *T. reesei* production organism, and the safety, in general, of microbial enzyme preparations used in food processing. Danisco summarizes corroborative evidence to support the safety of aminopeptidase Y enzyme preparation. Danisco discusses results of studies and concludes that aminopeptidase Y enzyme concentrate is not mutagenic or clastogenic. Danisco reports results from an unpublished 90-day oral toxicity study in rats using the aminopeptidase Y enzyme concentrate and determined a NOAEL of 1000 mg TOS/kg bw/d based on the results from the highest dose tested in the 90-day study. Based on the NOAEL from the 90-day study (1000 mg TOS/kg bw/d) and the estimated dietary exposure from the intended uses of the aminopeptidase Y enzyme preparation, Danisco estimates a margin of safety of 442.[5]

Danisco 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 aminopeptidase Y. Based on bioinformatic analyses using criteria recommended by FAO/WHO (2001), Codex Alimentarius Commission (2009), and JECFA (2016), Danisco reports no results using a window of 80 amino acids with over 35% homology or any exact matches using window of 8 amino acids. Based on the totality of the information available Danisco concludes that it is unlikely that oral consumption of aminopeptidase Y enzyme preparation from its intended uses will result in allergenic responses.

Based on the data and information summarized above, Danisco concludes that aminopeptidase Y 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 Danisco's notice concluding that aminopeptidase Y 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 lipase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing aminopeptidase enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

[5] FDA notes that the margin of safety stated by the notifier is based on unpublished safety studies and is corroborative of the published information regarding enzyme preparations used in food processing.

Conclusions

Based on the information that Danisco provided, as well as other information available to FDA, we have no questions at this time regarding Danisco's conclusion that aminopeptidase Y enzyme preparation is GRAS under its intended conditions of use. This letter is not an affirmation that aminopeptidase Y 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 001048 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2023.08.31 17:30:03 -04'00'

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