Koichiro Kojima
NAGASE & CO. LTD.
5-1 Nihonbashi-Kobunacho Chuo-ku
Tokyo 103-8355
JAPAN

Re: GRAS Notice No. GRN 001046

Dear Mr. Kojima:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001046. We received Nagase & Co. Ltd (Nagase)'s GRAS notice on December 1, 2021, filed it on April 25, 2022, and designated it as GRN 001046. Nagase submitted amendments to the notice on February 13, 2022, authorizing us to share the dossier with USDA, and on May 27, 2022, September 13, 2023, and April 12, 2023, providing additional information on the enzyme identity, suitability data, and information designated trade secret/confidential.[1]

The subject of the notice is collagenase enzyme preparation from genetically modified *Streptomyces violaceoruber* expressing a collagenase gene (collagenase enzyme preparation) for use as an enzyme at a level up to 10.9 mg Total Organic Solids (TOS)/kg in processing of beef jerky, marinated and injected meat, and sausage casings. The notice informs us of Nagase's view that these uses of collagenase enzyme preparation are 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. Nagase's notice provides information about the components in the collagenase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, collagenase is identified the Enzyme

[1] In the May 27, 2022 amendment, Nagase stated that information included in the original December 1, 2021 submission is considered trade secret/confidential. In the April 12, 2023 amendment, Nagase stated that the designation of trade secret/confidential was incorrect and to refer to the original submission for evaluation.

Commission Number 3.4.24.3;[2] the CAS Number for collagenase is 9001-12-1. Nagase states that the primary amino acid sequence of the collagenase consists of 865 residues and has a calculated molecular weight of 92.4 kDa.

Nagase states that the *S. violaceoruber* production organism is a non-pathogenic and non-toxigenic Gram-positive bacterium with a history of safe use in food processing. Nagase states that the *S. violaceoruber* pCol production strain was obtained by transformation of the recipient strain with a plasmid containing a promoter sequence from *S. avermitilis,* a collagenase gene from *S. violaceoruber* NBRC 15146, a terminator sequence from *S. cinnamoneus* and a thiostrepton antibiotic resistance gene selectable marker. The stability of the introduced DNA sequences was confirmed by measuring enzyme activity after fermentation of the production strain for several generations.

Nagase states that the collagenase enzyme preparation is manufactured by controlled fermentation of a pure culture of the *S. violaceoruber* production strain. After fermentation, the fermentation medium containing the enzyme is separated from the biomass by a series of filtration steps and sedimentation. The resulting collagenase enzyme concentrate is freeze dried and formulated with dextrin to a powdered collagenase enzyme preparation. Nagase states that the entire process is performed in accordance with current Good Manufacturing Practices and with raw materials that are food-grade. Nagase also states that none of the raw materials or processing aids used in the manufacturing process contain major allergens or are derived from allergenic sources.

Nagase has established food-grade specifications and states that the collagenase enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 11th edition, 2018), 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). Nagase provides data from analyses of six batches of collagenase enzyme preparation to demonstrate that the manufacturing acceptance criteria can be met. Nagase also provides data to confirm absence of potential mycotoxins, and presence of low levels of subsidiary lipase activity in the collagenase enzyme preparation.

Nagase states that the collagenase enzyme preparation is intended for use at a maximum level of 10.9 mg TOS/kg raw material catalyzes the selective hydrolysis of collagen in beef jerky, marinated and injected meat, and sausage casings, to assist in tenderization of these products. Nagase notes that the collagenase enzyme will be inactivated by a heating step during meat processing. Nagase estimates a maximum dietary exposure to collagenase enzyme preparation to be 0.068 mg TOS/kg body weight per day (mg TOS/kg bw/d) from all the intended uses, with the assumption that the added collagenase enzyme preparation will remain in the final food.[3]

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[2] https://iubmb.qmul.ac.uk/enzyme/EC3/4/24/3.html

[3] Nagase uses the Budget method to estimate dietary exposure to collagenase enzyme preparation based on a maximum use levels of 10.9 mg TOS/kg of liquid foods, and consumption of a maximum of 12.5 g of solid foods per kg body weight per day.

Nagase relies on published information that discusses the safety of the *S. violaceoruber* production organism and the safety of microbial enzyme preparations used in food processing. Nagase discusses results from a published ninety-day oral toxicity study (90-day) in rats using the collagenase enzyme concentrate. Nagase also summarizes corroborative unpublished toxicological studies using the powdered collagenase enzyme concentrate. These include tests conducted with bacterial cells to show that the collagenase is not mutagenic at the highest dose tested, both in the presence and absence of metabolic activation. Nagase also demonstrates that the collagenase enzyme concentrate is not clastogenic based on results from *in vitro* mammalian cell micronucleus test.

Nagase 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 collagenase. Based on bioinformatic analyses using criteria recommended by FAO/WHO (FAO/WHO, 2001; Codex Alimentarius, 2009; JECFA, 2016), Nagase reports no matches with greater than 35% identity using a window of 80 amino acids, and no exact matches using a window of 8 amino acids. Nagase also stated that no sequences were considered homologous with known allergens using a full sequence search with an E-value cut-off of 0.1. Based on the totality of the information available Nagase concludes that it is unlikely that oral consumption of collagenase will result in allergenic responses from its intended uses.

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

Use in Products under USDA Jurisdiction

As provided under 21 CFR 170.270, during our evaluation of GRN 001046, we coordinated with the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture. Under the Federal Meat Inspection Act, the Poultry Products Inspection Act, and the Egg Products Inspection Act, FSIS determines the efficacy and suitability of ingredients used in meat, poultry, and egg products, and prescribes safe conditions of use. Suitability relates to the ingredient's effectiveness in performing its intended technical effect and the assurance that the ingredient's use will not result in products that are adulterated or misleading for consumers.

FSIS completed its review and has no objection to the use of this collagenase enzyme preparation, subject of GRN 001046, as an enzyme at up to 10.6 mg TOS/kg raw material in the processing of beef jerky, marinated and injected meat, and sausage casings. Regarding labeling, meat or poultry products containing this collagenase enzyme preparation are required to be labeled in the ingredients statement with the common or usual name for the preparation's components of collagenase and dextrin.

Please contact Ms. Rosalyn Murphy-Jenkins at (301) 504-0879 or via email at Rosalyn.Murphy-Jenkins@usda.gov if you have questions regarding labeling. If you have any other questions, please contact Dr. Aaron Beczkiewicz by email at Aaron.Beczkiewicz@usda.gov or by phone at (314) 679-6821.

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 Nagase's notice concluding that collagenase 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 collagenase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing collagenase enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Nagase provided, as well as other information available to FDA, we have no questions at this time regarding Nagase's conclusion that collagenase enzyme preparation from genetically modified *S. violaceoruber* expressing a collagenase gene is GRAS under its intended conditions of use. This letter is not an affirmation that collagenase enzyme preparation from genetically modified *S. violaceoruber* expressing a collagenase gene 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 001046 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2023.06.22 18:19:36 -04'00'

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