Melvin S. Drozen
Keller and Heckman LLP
1001 G Street, NW
Suite 500 West
Washington, DC 20001

Re: GRAS Notice No. GRN 000908

Dear Mr. Drozen:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000908. We received the notice you submitted on behalf of Amano Enzyme Inc. (Amano) on January 15, 2020 and filed it on March 20, 2020. Amano submitted amendments to the notice on December 3, 2020, and January 5, 2021,[1] that provided additional information about the intended use, enzyme purification, carrier material (maltodextrin), literature search, enzyme activity, and dietary exposure.

The subject of the notice is acylglycerol lipase enzyme preparation produced by *Penicillium camemberti* genetically engineered to overexpress acylglycerol lipase (lipase enzyme preparation) at up to 2522 mg Total Organic Solids (TOS)/kg starting material for use as an enzyme in the production of enzyme-modified cheese (EMC) and processing of fats and oils. The notice informs us of Amano's view that the uses of lipase 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. Amano's notice provides information about the components in the lipase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, acylglycerol lipase is identified by the Enzyme Commission Number 3.1.1.23.[2] The enzyme, known by the systematic name glycerol-ester acylhydrolase, hydrolyzes glycerol monoesters of long-chain fatty acids.

[1] The January 5, 2021 amendment contained information that Amano designated confidential. The January 28, 2021 amendment included a revised version of the information from the January 5, 2021 amendment that was no longer designated confidential and stated that the notifier does not consider the information in either amendment to be confidential.
[2] https://www.qmul.ac.uk/sbcs/iubmb/enzyme/EC3/1/1/23.html

Amano states that the lipase is 305 amino acids in length with a corresponding molecular weight of 32.9 kDa.

Amano states that the *P. camemberti* production strain, AE-LGS, is non-pathogenic and non-toxigenic. It was derived from the *P. camemberti* recipient strain U-150,[3] which was UV irradiated to generate the orotidine-5'-phosphate decarboxylase deficient mutant. Amano describes the *P. camemberti* production strain was constructed by the integration of the native gene *mdlA* encoding acylglycerol lipase and gene *pyrG* encoding orotidine-5'-phosphate decarboxylase from *P. camemberti*. Amano states that the integration was confirmed by PCR and the stability of the integration was confirmed by monitoring continued lipase activity. Amano states that the final production strain does not contain any functional or transferable antibiotic resistance genes.

Amano states that lipase enzyme preparation is manufactured by submerged fed-batch fermentation of a pure culture of the production strain as described in GRN 000068; this information is incorporated by reference. An additional intermediate step in the filtration sequence includes subjecting the enzyme to heat and pH adjustment (pH 6.4-6.6). The resulting liquid enzyme concentrate is used as the test article for toxicity studies. After filtration, the liquid enzyme concentrate is treated with cold ethanol, and the precipitate is collected, dried, and standardized with maltodextrin.[4] Amano states that the entire process is performed in accordance with current good manufacturing practices and all materials used in fermentation and enzyme recovery are food-grade and safe and suitable for their intended use. Amano states that the fermentation media includes a soy-derived ingredient, and that the residues are removed during filtration. Amano states that the final lipase enzyme preparation does not contain any major food allergens based on results from targeted enzyme-linked immunosorbent assay (ELISA) analyses.

Amano has established food grade specification and states that the lipase 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). Amano provides data from analyses of three batches of lipase enzyme concentrate to demonstrate that the manufacturing specifications have been met

Amano intends to use lipase enzyme preparation at up to 2522 mg TOS/kg starting material, to hydrolyze mono- and di-acylglycerols to glycerol and free fatty acids in dairy and fats and oil processing. For dairy applications, the enzyme preparation will be added to cheese, cheese curd or fluid milk (at the initial stage of cheese production) to produce EMC for use in soups, snacks, or processed cheese blends. The lipase enzyme preparation will also be added during processing of fats and oils that are subsequently

[3] The notifier states that the recipient strain U-150 is designated by Amano as AE-LG and was used to produce the lipase enzyme preparation that is the subject of GRN 000068. We evaluated GRN 000068 and responded in a letter dated May 29, 2001, stating that we had no questions at that time regarding Amano's GRAS conclusion.

[4] As cited in 21 CFR 184.1444.

refined, bleached, and deodorized prior to use in foods. Amano notes that the lipase enzyme preparation will be denatured and/or removed during manufacture of these foods. However, in estimating dietary exposure, Amano assumes that all the lipase enzyme preparation will remain in the final food. Amano estimated dietary exposure to lipase enzyme preparation from dairy and fats and oils to be 3.8 mg TOS/kg body weight per day (mg TOS/kg bw/d).[5]

Amano relies on published information that discusses the safety of the *P. camemberti* production and donor organism and the safety of microbial enzyme preparations used in food processing, in general. Amano discusses cyclopiazonic acid, a mycotoxin of potential concern for some strains of *P. camemberti* under certain environmental conditions. However, Amano states that no mycotoxins, including cyclopiazonic acid, were detected in their lipase enzyme preparation. Further, Amano discusses safety of the lipase enzyme. Amano discusses results from published toxicological studies that did not show any treatment-related adverse effects up to the highest level of lipase enzyme concentrate tested. This includes a 90-day oral toxicity study in rats; the highest dose, equivalent to 1515 mg TOS/kg bw, was considered as the No-Observed-Adverse-Effect level (NOAEL). Amano calculates a margin of exposure to be 399 using the NOAEL and the estimated dietary exposure for the intended uses of the lipase enzyme.

Amano discusses publicly available literature as well as the conclusions of several organizations and working groups about the low risk of allergenicity posed by enzymes to address potential allergenicity due to lipase. Further, based on bioinformatic analyses, Amano reports that no match was found comparing the acylglycerol lipase against Structural Database of Allergenic Proteins in searches that included >35% homology over a sliding "window" of 80 amino acids and an exact match of 8 contiguous amino acids. Based on the totality of the information available, Amano concludes that it is unlikely that oral consumption of lipase enzyme will result in allergic responses.

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

Standards of Identity

In the notice, Amano states its intention to use lipase enzyme preparation in several food categories, including foods for which standards of identity exist, located in Title 21 of the Code of Federal Regulations. 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

[5] Amano estimated dietary exposure to lipase enzyme preparation based on consumption of 3 kg food per person per day and *all* food containing 76 mg TOS/kg food (based on the level of incorporation of EMC and enzyme-treated fats/oils in food).

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 Amano's notice concluding that lipase 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 lipase enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Amano provided, as well as other information available to FDA, we have no questions at this time regarding Amano's conclusion that lipase enzyme preparation lipase produced by produced by *Penicillium camemberti* genetically engineered to overexpress acylglycerol lipase is GRAS under its intended conditions of use. This letter is not an affirmation that lipase enzyme preparation produced by *Penicillium camemberti* genetically engineered to overexpress acylglycerol lipase 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 000908 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2021.02.05 14:05:37 -05'00'

Susan Carlson, Ph.D.
Director
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