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January 14, 2020

Via FedEx

Paulette Gaynor, Ph.D.
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Campus Drive
College Park, MD 20740

Re: GRAS Notification for Amano's Lipase from *Penicillium camemberti*; Replaces GRN 866

Dear Dr. Gaynor:

We respectfully submit the attached (new) GRAS notification (in electronic format, *i.e.*, CD)[1] on behalf of our client, Amano Enzyme, Inc. (Amano) for a lipase enzyme preparation derived from *Penicillium camemberti* for use as a processing aid for (1) dairy processing (*i.e.*, the production of enzyme-modified cheese) and (2) fats and oil processing (*i.e.*, the production of dietary ingredients derived from fats and oils including, but not limited to, gamma linolenic acid (GLA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA)). The enclosed GRAS notification replaces GRN 866 (which Amano asked FDA to stop reviewing) and provides detailed information related to the intended uses, manufacturing, and safety of the enzyme when derived from a self-cloned derivative of *P. camemberti* U-150 that produces higher yields of the enzyme than the original strain.

The enclosed new GRAS notice conforms with FDA's administrative preference of having Amano's GRAS conclusion regarding the intended uses of the lipase derived from the self-cloned strain of *P. camemberti* (designated internally by Amano as AE-LGS) addressed in a separate submission from Amano's GRAS conclusion regarding the same intended uses of the lipase from the non-GM strain (designated internally by Amano as strain AE-LG) as opposed to setting forth the GRAS conclusion for the intended uses of the enzyme (manufactured using

[1] All electronic files included in this submission have been checked and found to be virus free.

either production organism) in a single GRAS notice, *i.e.*, GRN 866.[2] Amano withdrew GRN 866 (which covers the enzyme derived from *P. camemberti* U-150 and, alternatively, from the self-cloned derivative) in deference to FDA's view that distinctions with respect to the identities of the alternative production organisms for the acylglycerol lipase make it impossible from an administrative perspective for FDA to provide a "no questions" letter for GRN 866 of appropriate clarity and transparency suitable to the voluntary GRAS notification program.

No additional information is provided in the enclosed GRAS notice as compared to GRN 866. The only significant difference between the enclosed GRAS notice and GRN 866 is in Part 1.3 (Name of the notified substance), which is a short paragraph that was rewritten so that the enclosed new GRAS notice provides Amano's GRAS conclusion for the intended uses of the lipase derived only from the self-cloned strain of *P. camemberti*, as opposed to also being produced by the non-GM strain. Additionally, Parts 2.2 (Description of the method of manufacture) and 6.1 (Safety of *Penicillium camemberti* AE-LGS) were slightly revised to delete a few references to the production and safety of the enzyme as produced by the non-GM strain. Further, as nomenclature to distinguish between the alternative production organisms, *i.e.*, AE-LG and AE-LGS for the non-GM and self-cloned strains, respectively, is not used outside of Amano, and the self-cloned strain does not contain any foreign DNA, the proposed common or usual name of the notified substance, as set forth in Part 2.1(a), remains "Lipase from *Penicillium camemberti*," which is the same name as in GRN 68 except for an updated spelling (*i.e.*, the species name of the production organism is now spelled with a single-i, rather than as *Penicillium camembertii*). Strain designation is not meaningful for this production organism because neither *P. camemberti* U-150 nor the self-cloned strain have been submitted to any public repository and both are in Amano's sole possession.

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[2] GRN 866 was submitted to FDA on May 30, 2019 and filed by FDA on August 1, 2019. On October 24, 2019, FDA alerted us of unspecified questions about GRN 866. In an October 31, 2019 teleconference, followed by a November 14, 2019 email, FDA did not question Amano's GRAS conclusion, but informed us of the need for "two separate submissions, one for each of the distinctly derived enzyme preparations." By email on November 19, 2019, we asked FDA to reconsider whether an amendment providing a concise summary of the submission would enable FDA to move forward with GRN 866. FDA informed us by telephone on December 17, 2019 that it will not be possible to cover the acylglycerol lipase enzyme when produced by both the non-GM strain and the self-cloned strain in a single GRAS notice and that FDA would deem a replacement submission – to resolve the perceived clarity issues by omitting intended uses of acylglycerol lipase enzyme preparation produced from the non-GM strain of *P. camemberti* U-150 to be a new notice and, as such, a new GRAS submission process needs to be followed.

We hope that FDA will be able to file and review this voluntary GRAS notice expediently since there are no substantive differences from GRN 866, which was filed two months after delivery to FDA and was under scientific review for 84 days before FDA provided notice of questions unrelated to the safety of the intended uses of the enzyme. Thank you for your attention to this matter.

Sincerely,

Melvin S. Drozen

Enclosure

GRAS Notice for Lipase Derived From *Penicillium camemberti*

Prepared for: U.S. Food and Drug Administration
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
5100 Paint Branch Parkway
College Park, MD 20740-3835

Prepared by: Keller and Heckman LLP
1001 G Street, NW
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Washington, DC 20001

Date: January 14, 2020

Table of Contents

GRAS NOTICE FOR LIPASE DERIVED FROM *PENICILLIUM CAMEMBERTI*

SUBMITTED BY AMANO ENZYME, INC.

Part 1 – Signed statements and certification

1.1 Applicability of 21 C.F.R. part 170, subpart E

We submit this GRAS notice in accordance with 21 C.F.R. part 170, subpart E.

1.2 Name and address of the notifier

Company: Amano Enzyme, Inc.
Name: Mr. Tomonari Ogawa (Director, Quality Assurance Division)
Address: 2-7, 1-Chome, Nishiki, Naka-ku, Nagoya, Japan 460-8630
Phone: 81 (0) 568.21.4044
Fax: 81 (0) 568.26.6160

1.3 Name of the notified substance

The notified substance is lipase derived from *Penicillium camemberti*. The production organism was derived by self-cloning from *P. camemberti* U-150, which is the original production organism for this lipase. *P. camemberti* U-150 is a non-genetically modified (GM) strain, designated internally by Amano as strain AE-LG, as described in in Amano's GRN 68. The new, GM strain is designated as AE-LGS. No strain designations have been publicly assigned for either the GM or non-GM strains of the production organism. The intended uses of the lipase are expanded as compared to the intended uses of the lipase described in GRN 68. Amano's GM and non-GM *P. camemberti* enzyme preparations contain dextrin, which is used as a diluent and is GRAS affirmed at 21 C.F.R. 184.1277.

1.4 Applicable conditions of use of the notified substance

1.4(a) Foods in which the substance is to be used

The lipase is intended for use as a processing aid for dairy processing (*i.e.*, the production of enzyme-modified cheese) and fats and oil processing (*i.e.*, the production of ingredients derived from fats and oils including, but not limited to, gamma linolenic acid (GLA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA)). By comparison, GRN 68 describes a narrower intended use of the lipase derived from *P. camemberti* AE-LG for use only in the production of ingredients derived from fats and oils, rather than also covering use of the enzyme for dairy processing as described in this GRAS notice. *See* GRN 68, Section 1.iii ("Conditions of Use") at page 000004.

1.4(b) Levels of use in such foods

Lipase is a processing aid. It has no activity in the finished food due to the denaturation of the enzyme by heat treatment during processing of the food or food ingredient made using the lipase. Some manufacturing uses of the lipase additionally entail depletion of the substrate and later food processing steps, including reduction of water activity and altering the pH, which prohibit enzyme activity in the finished food or food ingredient. Further, the inactivated enzyme – which is water soluble – is removed from foods or food ingredients such as GLA, DHA, and EPA that are produced with a washing step that

follows the enzyme treatment in the manufacturing process. Nevertheless, in calculating the estimated daily intake (EDI) of the lipase, it is assumed that the maximum amount of lipase used in the applications of interest (*i.e.*, dairy processing and fats/oils processing) remains in the food ingredient when it is consumed.

The use level of the lipase is expressed in terms of the amount of Total Organic Solids (TOS) of *P. camemberti* enzyme preparation, in milligrams (mg), that are added to the fat substrate and may potentially be present in each kilogram (kg) of raw material (*i.e.*, component of the finished food). The TOS is calculated by subtracting the weight of ash from the weight of the dry matter comprising the enzyme preparation. Ash in the enzyme preparation (*e.g.*, mineral salts used in the fermentation) generally does not exceed a few percent. Any food material that contains monoglycerides and diglycerides could be suitable for use as a substrate for the lipase.

The intended use of Amano's lipase derived from *P. camemberti* in dairy processing and fats/oils processing, with representative examples of substrates, is described in greater detail as follows.

Dairy processing

Lipase is added to emulsified cheese homogenates (the raw material), along with animal or microbial enzymes, at levels ranging from **25 to 2,522 mg TOS/kg raw material** during the incubation stage in making enzyme modified cheese (EMC). A subsequent heat treatment of the EMC at 90°C for 15 minutes inactivates the lipase. The EMC may be used as an ingredient in foods such as soup, snack foods, and processed cheese.

Fats and oil processing

Lipase is added at levels ranging from **50 to 252 mg TOS/kg raw material** (*i.e.*, edible fats and oils, such as shea oil, fish oil, or borage oil) prior to refining (deacidification and dehydration), bleaching, and deodorizing. The lipase hydrolyzes diglycerides into monoglyceride and/or fatty acids but does not react with triglycerides. The bleaching and deodorizing steps involve heat treatments that are expected to denature the lipase. Fat and oil ingredients made using the lipase may be used as an ingredient in bread, pastry, and confections.

1.4(c) Purpose for which the substance is to be used

Amano's *Penicillium camemberti* enzyme preparation (acylglycerol lipase or lipase) catalyzes the hydrolysis of ester bonds between fatty acids and glycerols in monoglycerides and diglycerides that are present in edible fats and oils of plant and animal origin.

In dairy processing, the lipase hydrolyzes milk fat to generate free fatty acids that enhance the flavor of EMC.

In fat and oil processing, the lipase-modified fats and oils have a low diglyceride content, exhibit rapid crystallization rates, are less susceptible to hydrolysis, and have unchanged triglyceride compositions.

1.4(d) Description of the population expected to use the substance

Amano's *P. camemberti* enzyme preparation is expected to be consumed by any population that consumes enzyme modified cheese and/or foods and beverages that may contain ingredients such as DHA, EPA, and GLA.

1.5 Basis for the GRAS determination

The statutory basis for our conclusion of GRAS status is through scientific procedures in accordance with 21 C.F.R. §§ 170.30(a) and (b).

1.6 Exclusion from premarket approval

The notified substance is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act (FD&C Act) based on our conclusion that the notified substance is GRAS under the conditions of its intended use.

1.7 Availability of data and information

If the Food and Drug Administration (FDA) asks to see the data and information that are the bases for our conclusion of GRAS status, either during or after FDA's evaluation of our notice, we agree to make the data and information available to FDA. Further, upon FDA's request, we will allow the Agency to review and copy the data and information during customary business hours at the above address and will provide FDA with a complete copy of the data and information, either in an electronic format that is accessible for the Agency's evaluation, or on paper.

1.8 Applicability of FOIA exemptions

This GRAS notice does not contain confidential business information (CBI) exempt from disclosure under the Freedom of Information Act per 5 U.S.C. § 552(b)(4).

1.9 Certification

We certify that, to the best of our knowledge, our GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as

favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of the substance.

[redacted] Jan 14, 2020

Name: Mr. Tomonari Ogawa Date
Title: Director, Quality Assurance Division

Please address correspondence to Amano's counsel:

Melvin S. Drozen
Keller and Heckman LLP
1001 G Street, N.W., Suite 500 West
Washington, DC 20001
Phone: (202) 434-4222
Email: drozen@khlaw.com

Part 2 – Identity, method of manufacture, specifications, and physical or technical effect

2.1 Scientific data and information that identifies the notified substance

The trade name for Amano's *Penicillium camemberti* enzyme preparation, described below, is Lipase GS "Amano" 250G. The product is a low odor, water soluble granule with a color that is light yellowish brown to light brown. Food grade dextrin is used as a carrier for the enzyme in accordance with good manufacturing practice (GMP). Dextrin is affirmed as GRAS at 21 C.F.R. § 184.1277. The lipase enzyme component is extracted and purified from the submerged fermentation and processing of a stable non-pathogenic strain of *P. camemberti* AE-LGS (a strain number used internally by Amano) that has been shown to be non-toxic under the growth conditions for enzyme production. *P. camemberti* AE-LGS is a self-cloned derivative of *P. camemberti* AE-LG (a strain number used internally by Amano). The enzyme preparation derived from *P. camemberti* AE-LG (trade name Lipase G "Amano" 50) is described in GRN 68.[1] Lipase GS "Amano" 250G and Lipase G "Amano" 50 are equivalent.

More detailed information regarding the identity of the notified substance is provided as follows:

2.1(a) Common or usual name

Lipase from *Penicillium camemberti*.

2.1(b) International Union of Biochemistry and Molecular Biology (IUBMB) Enzyme Nomenclature Chemical name

Acylglycerol lipase

2.1(c) IUMB Number

EC 3.1.1.23

2.1(d) Chemical Abstracts Service Registry Number (CASRN)

9040-75-9

2.1(e) European INventory of Existing Commercial chemical Substances (EINECS) Number

679-517-4

[1] The currently accepted spelling of the name of the production organism is *Penicillium camemberti*, with a single-i, rather than *Penicillium camembertii*, with a double-ii.

2.1(f) Synonyms

Monoacylglycerol lipase; monoacylglycerolipase; monoglyceride lipase; monoglyceride hydrolase; fatty acyl monoester lipase; monoacylglycerol hydrolase; monoglyceridyllipase; monoglyceridase

2.1(g) Enzyme specificity

Amano's *Penicillium camemberti* enzyme preparation (acylglycerol lipase or lipase) catalyzes the hydrolysis of ester bonds between fatty acid and glycerol in monoglycerides and diglycerides that are present in edible fats and oils of plant and animal origin, releases fatty acids and glycerols. The enzyme does not affect triglycerides.

2.1 (h) Molecular mass and amino acid sequence

The full amino acid sequence of lipase derived from *Penicillium camemberti* is provided in **Figure 1** below.

Figure 1. Amino acid sequence of lipase from *Penicillium camemberti*

```
1 MRLSFFTALS AVASLGYALP GKLQSRDVST SELDQFEFWV QYAAASYYEA DYTAQVGDKL 60
61 SCSKGNCPEV EATGATVSYD FSDSTITDTA GYIAVDHTNS AVVLAFRGSY SVRNWVADAT 120
121 FVHTNPGLCD GCLAELGFWS SWKLVRDDII KELKEVVAQN PNYELVVVGH SLGAAVATLA 180
181 ATDLRGKGYP SAKLYAYASP RVGNAALAKY ITAQGNNFRF THTNDPVPKL PLLSMGYVHV 240
241 SPEYWITSPN NATVSTSDIK VIDGDVSFDG NTGTGLPLLT DFEAHIWYFV QVDAGKGPGL 300
301 PFKRV
```

From the above sequence, the molecular mass of the enzyme protein is calculated to be 32.9kDa.

Additional information about acylglycerol lipase derived from *Penicillium camemberti* is available in protein databases such as BRENDA (https://www.brenda-enzymes.org/) and UniProt (https://www.uniprot.org/).

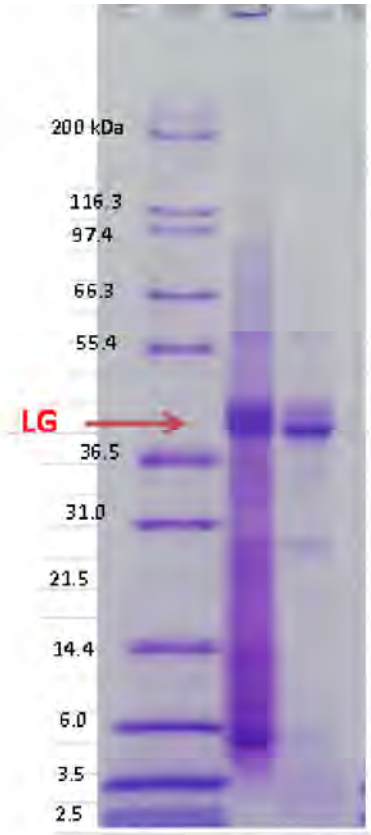
2.1(i) Equivalence of lipase from *P. camemberti* AE-LGS to lipase from *P. camemberti* AE-LG

P. camemberti AE-LGS is a self-cloned derivative of *P. camemberti* AE-LG (as described in detail in item 2.1(k) below). Based on the analyses that follow, the lipase from Amano's GM strain (*P. camemberti* AE-LGS) is deemed to be identical to the lipase from Amano's non-GM strain (*P. camemberti* AE-LG) that is the subject of GRN 68.

Comparative analysis by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) shows banding patterns that support no significant differences in protein expression between the self-cloned strain, *P. camemberti* AE-LGS (LG-Y69-001) and

Amano's non-GM strain, *P. camemberii* AE-LG (LG-Y67-003) that is the subject of GRN 68. See **Figure 2** below.

Figure 2. SDS-PAGE analysis of lipases derived from *P. camemberti* AE-LG (LG-Y67-003) and *P. camemberti* AE-LGS (self-cloned from *P. camemberti* AE-LG) (LG-Y69-001).



From the left

Maerker, LG-Y67-003, LGS-Y69-001

Gel: Super sep ACE (WAKO pure chmical)
10-20% 17well
3000v 15A Conditions of electrophoresis

Marker: Marker12 Unstained standard (Invitrogen)
Eight-tube for PCR

↓←20μL 10000 U-LV/mL

↓←25μL

↓←55μL

↓←Voltex

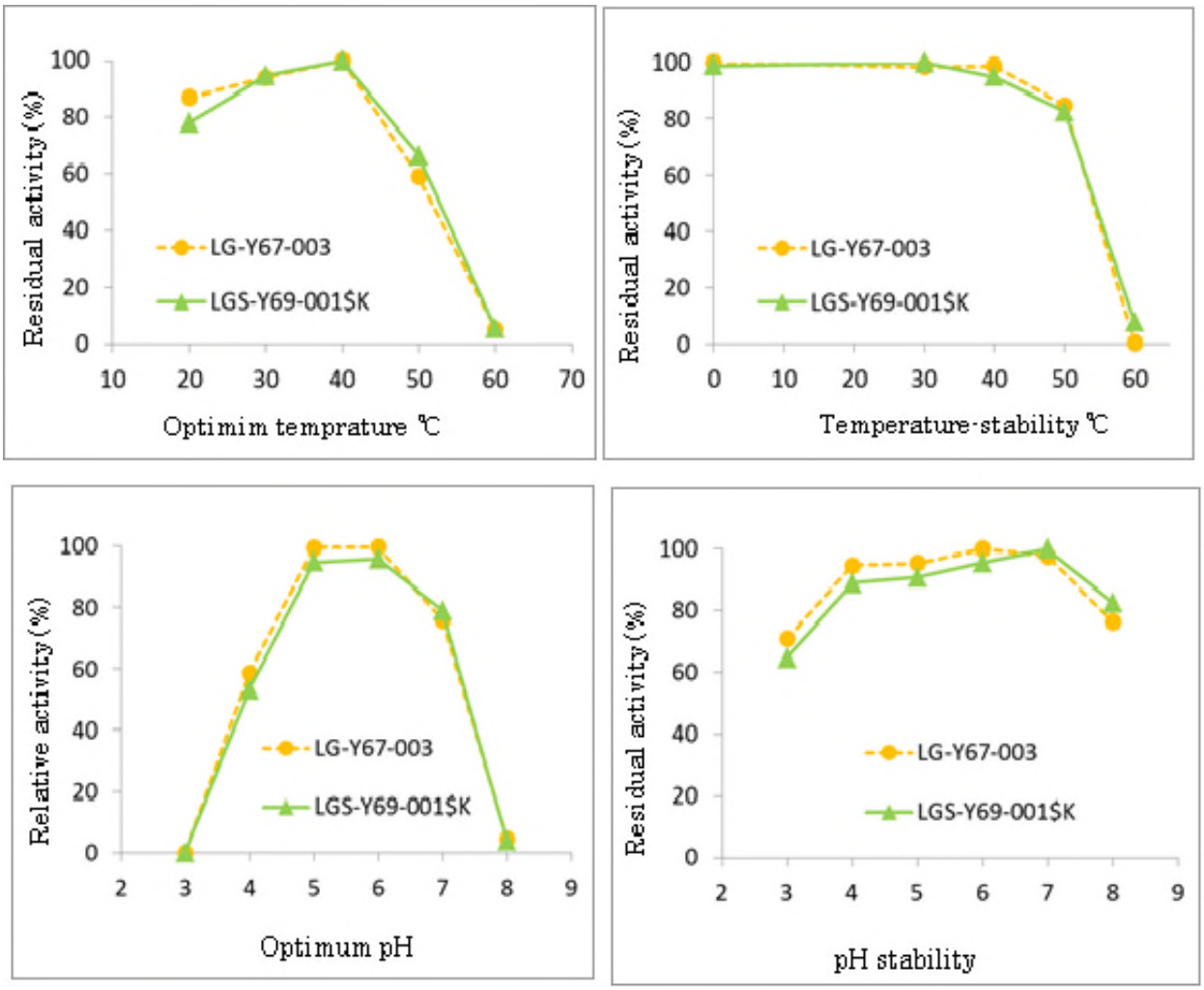
↓←99℃ 10 min Thermal cycler

10μL electrophoresis

LGS-Y69-001:
Production strain is self cloned microorganism

Optimum temperature, temperature stability, pH stability, and optimum pH were equivalent for the lipase from Amano's GM strain (*P. camemberti* AE-LGS) as compared to the lipase from Amano's non-GM strain (*P. camemberti* AE-LG) that is the subject of GRN 68. See **Figure 3** below.

Figure 3. Optimum temperature, temperature stability, pH stability, and optimum pH of lipases derived from *P. camemberti* AE-LG (LG-Y67-003) and *P. camemberti* AE-LGS (self-cloned from *P. camemberti* AE-LG) (LG-Y69-001SK).



TOS contents were equivalent for the lipase from Amano's GM strain (*P. camemberti* AE-LGS) as compared to the lipase from Amano's non-GM strain (*P. camemberti* AE-LG) that is the subject of GRN 68. Lipase derived from Amano's GM strain is of a higher purity than that derived from non-GM strains as indicated by activity (U/g) assays. See **Table 2** below.

Table 2. Comparison of lipases derived from *P. camemberti* AE-LG ("LG (Non-GM)") and *P. camemberti* AE-LGS (self-cloned from *P. camemberti* AE-LG; "LGS (GM)").

	LGS (GM)	**Test article (90 day toxicity study)**	**LG (Non-GM)**			
Batch No.	**LGS-Y69-001**	**LGK-000602**	**LG-Y64-002**	**LG-Y65-001**	**LG-Y65-002**	**Mean**
Ash (%)	2.52	22.2*	1.8	3.1	2.6	2.5
Water (%)	4.9	2.04	4.4	4.3	5.1	4.6
TOS (%)	92.58	75.76	93.80	92.60	92.30	92.9
Activity (Units/g)	913,000	100,222	337,000	140,000	304,000	260,333
Units/mg TOS	986.2	132.3	359.3	151.2	329.4	279.9

* The ash content of the test article samples was high because the liquid before alcohol precipitation was freeze-dried.

2.1(j) Taxonomic classification of the production strains

Super Kingdom	Eukaryote
Kingdom	Fungi
Phylum	Ascomycota
Order	Eurotiales
Class	Eurotiomycetes
Family	Trichocomaceae
Genus	*Penicillium*
Species	*camemberti*
Strain	AE-LG (U-150) and AE-LGS (not registered)

2.1(k) Information regarding the source of the notified substance

The lipase production strain, *Penicillium camemberti* AE-LGS, was obtained via self-cloning from *P. camemberti* U-150 (designated internally by Amano as strain AE-LG).

FDA has issued a May 21, 2009 letter indicating that the Agency has "no questions" regarding the GRAS status of lipase derived from *P. camemberti* U-150 for use in the production of ingredients derived from fats and oils as described in Amano's GRN 68. (The intended uses of the lipase in this GRAS notice are expanded to include (1) dairy processing (i.e., the production of enzyme-modified cheese) and (2) fats and oil processing (*i.e.*, the production of ingredients derived from fats and oils including, but not limited to, gamma linolenic acid (GLA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA)), which is described in GRN 68.)

P. camemberti AE-LGS contains two genes from *P. camemberti* AE-LG: (1) native lipase gene, mdlA, and (2) marker gene, pyrG (orotidine-5'-phosphate decarboxylase) for non-toxic nutritional selection of transformed cells with amplified mdlA genes.

Using a pyrG-deficient strain derived from UV treatment of *P. camemberti* AE-LG as the host, transformation was conducted by the protoplast method to achieve the target transformant. The DNA fragments mdlA and pyrG were incorporated into the host genome through non-homologous recombination. Transformants were grown on a plate spiked with brilliant green and monoolein, and primary screening was conducted based on the Halo size. Strains with high lipase productivity were selected.

2.2 Description of the method of manufacture

The lipase enzyme preparation is manufactured under controlled fermentation conditions using a pure culture of *Penicillium camemberti* AE-LGS (Amano's internal designation for the strain derived by self-cloning of *P. camemberti* U-150, which is designated internally as strain AE-LG). Production of the enzyme complies with FDA's current good manufacturing practice (CGMP), hazard analysis, and risk-based preventive controls (HARPC) for human food regulations set forth in 21 C.F.R. Part 117. A detailed description of the manufacturing process, which was provided in GRN 68 and is incorporated herein, reflects the current process with the exception of minor changes made to improve process efficiency. *See* GRN 68 at Appendix I, located at page 13 of GRN 68.[2] As compared to the previous (conventional) technology, the hours of growth in the main fermentation step have decreased and the enzyme is now harvested at a slightly lower optical density. Additionally, the filtration process was optimized. Specifically, a new course-filtering step was added and the microfiltration steps now employ only the most ideal grade of diatomaceous earth.

Each raw material used in manufacturing lipase derived from *Penicillium camemberti* has an appropriate FDA regulatory status for its intended use and is of a grade suitable for use in producing food. In the future, should Amano choose to modify the raw materials or

[2] "Specifically identified data and information" are incorporated into Amano's current GRAS notice by reference to GRN 68 as permitted under 21 C.F.R. § 170.215 ("Incorporation into a GRAS notice"). Amano's GRN 68 is publicly available on FDA's GRAS notice inventory at https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=68 and also remains in CFSAN's files as required for incorporation by reference under Section 170.215.

processing aids used in the production of the lipase enzyme preparation, the Company will ensure that all such components are safe and suitable for their intended use.

2.3 Specifications of the food-grade material

Specifications for the lipase enzyme are in keeping with the requirements of the Food Chemicals Codex (11th Edition) and Joint FAO/WHO Expert Committee on Food Additives (JECFA 2006) monographs for enzyme preparations used in food processing. **Table 3** below identifies the specifications for the lipase enzyme preparation.

Table 3. Specifications for the lipase enzyme preparation.

Item	**Requirement**	**Lot No. LGSQ0552101GK**	**Lot No. LGSQ0750501GK**	**Lot No. LGSQ1151401GK**
Lead	Not more than 5 mg/kg	0.042 mg/kg	0.037 mg/kg	0.047 mg/kg
Salmonella sp.	Negative, Not detected (ND), in 25 grams	ND in 25g	ND in 25g	ND in 25g
Total coliforms	Not more than 30 CFU/g	<10 CFU /g	<10 CFU /g	<10 CFU /g
Escherichia coli	Negative, Not detected (ND), in 25 grams	ND	ND	ND
Antimicrobial activity	Negative	Negative	Negative	Negative
Mycotoxins	No significant levels*	None detected	None detected	None detected

* Although nonpathogenic and nontoxigenic microorganisms are normally used in the production of enzymes used in food processing, several fungal species traditionally used as sources of enzymes are known to include strains capable of producing low levels of certain mycotoxins under fermentation conditions conducive to mycotoxin synthesis. According to JECFA specifications, the enzyme preparations derived from such fungal species should not contain toxicologically significant levels of mycotoxins that could be produced by these species. There are no concerns for mycotoxins in Amano's lipase derived from *P. camemberti*. *See* Part 6.2 below mycotoxin screening of Lipase GS "Amano" 250G and page 6 of GRN 68 regarding mycotoxin screening of Lipase G "Amano" 50, which is incorporated by reference to this GRAS notice. Testing of Lot Nos. LGSQ0552101GK, LGSQ0750501GK, and LGSQ1151401GK showed no mycotoxins were detected at the respective limits of detection listed in Figure 4 below.

2.4 Data and information bearing on the physical or other technical effect

The mode of action of the enzyme is presented at Part 2.1(g) above. This GRAS notice does not otherwise include data and other information bearing on a physical or other technical effect because such data and other information are not necessary to demonstrate safety.

Part 3 – Dietary exposure

As described above in Part 1.4(b), the lipase enzyme preparation is used at levels ranging from 25 to 2,522 mg TOS/kg enzyme-modified cheese (EMC) and 50 to 252 mg TOS/kg fat/oil ingredient.

For calculating the Estimated Daily Intake (EDI) of the lipase enzyme preparation, it is assumed that the maximum potential use levels described in Part 1.4(b) are always employed for each application. Furthermore, the calculation takes into account the expected level of each raw material (*i.e.*, ingredient using Amano's lipase) in the finished food, and it is assumed that all the TOS will end up in the final food product. The maximum levels of TOS in finished foods are tabulated below.

Table 4. Maximum level of TOS per kilogram of finished food.

Application	Raw Material (RM)	Maximum Recommended Use Level (mg TOS/kg RM)	Final Food	Ratio RM/Final Food	Maximum Level in Final food (mg TOS/kg Food)
Dairy Processing	Enzyme Modified Cheese	2522	Soup, snack foods, processed cheese	0.02	50
Fats and Oil Processing	Edible fats and oils	252	Bread, pastry, and confections	0.3	76

Although the maximum possible level of Amano's lipase would be 50 mg TOS kilogram in foods made with EMC produced using acylglycerol lipase, it is assumed that all foods and beverages contain 76 mg TOS per kilogram of food (which corresponds to the maximum level in bread, pastry, and confections, *i.e.*, the highest level for any food). Based on this exaggerative assumption, and applying FDA's standard assumption that an average daily diet consists of 3 kilograms (kg) of food per day (including 1.5 kg of beverages) and body weight of 60 kg, the EDI for Amano's lipase enzyme preparation is calculated to be:

3 kg/p/day x 76 mg TOS/kg food ÷ 60 kg bw = **3.8 mg TOS/kg bw/day.**

This calculated EDI is highly conservative because of the following exaggerative assumptions:

- All producers of the above mentioned foods use ingredients made with the acylglycerol lipase from *Penicillium camemberti*;
- All producers employ the HIGHEST use level of lipase per application;
- All foods contain the HIGHEST theoretical amount of TOS found in any food;

• The amount of TOS does not decrease as a result of the food production process;

• A person consumes only foods containing the calculated theoretical amount of TOS daily over the course of a lifetime.

- A person consumes 3 kg of solid food per day (which potentially contains ingredients made with acylglycerol lipase), rather than 1.5 kg of solid food and 1.5 kg of beverages (which are not expected to contain ingredients made with acylglycerol lipase).

Part 4 – Self-limiting levels of use

This part does not apply

Part 5 – Experience based on common use in food before 1958

This part does not apply

Part 6 – Narrative

The information provided in Part 2 above on the identity, manufacturing process, and specifications of the lipase derived from *Penicillium camemberti* support Amano's view regarding the equivalence of the lipase derived from a genetically modified strain of *P. camemberti* (*i.e.*, Lipase GS "Amano" 250G derived from *P. camemberti* AE-LGS, a self-cloned derivative of *P. camemberti* AE-LG) to the lipase from the non-genetically modified strain (*i.e.*, Lipase G "Amano" 50 derived from *P. camemberti* AE-LG as described in GRN 68). The information regarding Lipase GS "Amano" 250G in Part 2 of this GRAS notice and the information in GRN 68 regarding Lipase G "Amano" 50, together with the dietary exposure estimate provided in Part 3 above and the information provided in this section on the safety of the production organisms and the safety of the enzyme, including its lack of allergenic and toxigenic potential, provide a basis for Amano's determination that its lipase enzyme preparation derived from *Penicillium camemberti* is GRAS for use in dairy processing and fats/oil processing as discussed in detail below.

6.1 Safety of *Penicillium camemberti* AE-LGS

Although the production organism is not present in the lipase enzyme preparation, Amano follows the best practice of using nonpathogenic organisms for the production of enzymes intended for use in food applications. *Penicillium camemberti* is well known for its long history of use as a secondary cheese culture for mold surface-ripened cheeses, where it can be used to give a white felt on the surface of Coulommier and Camembert type cheese, as reported by G. Stanley in Encyclopedia of Food Sciences and Nutrition (Second Edition), 2003. A literature search for published reports of health concerns associated with *P. camemberti* (conducted January 3, 2020) indicates no concerns for pathogenicity and possibly a low level of potential concern for workplace allergenicity, not related to dietary exposure. In this regard, as discussed at page 9 of GRN 68, Marchisio et al. (1999) reported a possible case of extrinsic allergic alveolitis caused by exposure of an employee in a salami manufacturing facility to *P. camemberti* by inhalation. After Amano's GRN 68 was provided to FDA in 2001, Merget et al. (2008) reported the details of an employee who experienced progressive work-related asthma, rhinitis, and contact urticaria after contact with mold-cured dry sausages and had specific antiglobulin E antibodies to *P. camemberti*. The circumstances and route of exposure for the reported cases of allergic reactions in workers after repeated contact with high levels of *P. camemberti* used in finishing dried sausages do not raise safety concerns for dietary exposure to low levels of an extracellular lipase enzyme preparation derived *P. camemberti* and not containing the production organism. As discussed in Part 6.3 below, there are no allergenicity concerns for the lipase enzyme preparation.

While *P. camemberti* strains AE-LG (referred to as "U-150" in GRN 68) and AE-LGS (the self-cloned derivative of AE-LG) are not specifically known to have been used in cheese or dry sausage production, the fact that other *P. camemberti* strains have a long history of use in food production with no reports of infection supports the conclusion that the lipase production strains are not pathogenic. Further, a study showed no concerns for pathogenicity based on the histopathology analysis of mice 14 days after the injection into the tail vein of 10^2, 10^4, or 10^6 viable spores of *P. camemberti* AE-LG. *See* page 6 of

GRN 68, discussing the results of the pathogenicity testing reported by Kondo et al. (1994).

6.2 Safety of enzyme preparation from *Penicillium camemberti*

The pivotal safety data in concluding the intended use of Amano's lipase enzyme preparation is GRAS are from a 90-day toxicological study of Lipase G Amano 50 (LGK) in rats reported by Kondo et al. (1994), which was provided to FDA in GRN 68 (incorporated here by reference). No adverse toxicological data were located in an updated review of the toxicological literature conducted on August 23, 2018, aside from reports – discussed briefly below – which indicate that cyclopiazonic acid (CPA) is a mycotoxin of potential concern for some strains of *Penicillium camemberti* under certain environmental conditions.

Le Bars (1979) reported that each of 20 strains of *P. camemberti* isolated from cheese produced CPA at levels greatly dependent on the strain and environmental parameters (medium, temperature, and incubation time). Císarová M et al. (2012) reported that only 2 of 14 strains of *P. camemberti* isolated from samples of 20 different cheeses did not produce CPA under any conditions of the study and that production of CPA by the other 12 isolates varied by strain and was dependent on incubation temperature. No published data were located regarding production of CPA by *P. camemberti* U-150.

While there are no reports of illness linked to consumption of cheese with CPA produced by *P. camemberti*, acute toxicity is a recognized hazard for oral exposure to CPA. *See* PubChem, Open Chemistry Database, Compound Summary for CID 54682463, *available at* https://pubchem.ncbi.nlm.nih.gov/compound/cyclopiazonic_acid (accessed July 24, 2018). The toxic potency of CPA is moderate to high, *i.e.*, LD50 Rat oral 36 mg/kg and LD50 Mouse oral 64 mg/kg (*see* Lewis, R.J. *Sax's Dangerous Properties of Industrial Materials.* 9th ed. Volumes 1-3. New York, NY: Van Nostrand Reinhold, 1996., p. 973). Only one incident of human toxicity, however, has been potentially linked to the consumption of food possibly containing CPA. Namely, Rao and Husain (1985) reported on the detection of *Aspergillus flavus* and *Aspergillus tamarii* (two fungi known to produce CPA) in kodo millet associated with 'kodua poisoning' in humans and cattle in India. The possible link of human illness to CPA potentially present in food contaminated with two fungi that are not closely related to *P. camemberti* is not directly relevant to the safety analysis of the enzyme preparation derived from *P. camemberti*.

Based on an exhaustive review of the published research on CPA, Ostry et al. (2018) concluded:

> CPA is a potentially serious mycotoxin and its toxicity to several animal species warrant further chronic toxicity and carcinogenicity studies. The results of chronic toxicity and carcinogenicity studies, recent consumption data, and the occurrence of CPA in foodstuffs are required for the assessment of toxicity severity and estimation of human dietary exposure and health risk assessment.

CPA was not detected in the enzyme preparations derived from *P. camemberti* AE-LG (*i.e.*, U-150), as reported in GRN 68, or in *P. camemberti* AE-LGS, which was analyzed for a broad range of mycotoxins by high pressure liquid chromatography with fluorescence detection (HPLC/FD), liquid chromatography- tandem mass spectrometry (LC-MS/MS), or solid phase extraction (SPE) with LC-MS/MS with the results set forth in **Figure 4** below.

Figure 4. Mycotoxin screening of enzyme preparation derived from *Penicillium camemberti* AE-LGS.

Mycotoxin	**Detection Method**	**Result***
Aflatoxin B1	HPLC/FD	<0.2 µg/kg
Aflatoxin B2	HPLC/FD	<0.2 µg/kg
Aflatoxin G1	HPLC/FD	<0.2 µg/kg
Aflatoxin G2	HPLC/FD	<0.2 µg/kg
Total Aflatoxin	NA	<0.8 µg/kg
Ochratoxin	HPLC/FD	<0.5 µg/kg
Deoxynivalenol (DON)	LC-MS/MS	<10.0 µg/kg
Fusarium (HT-2)	LC-MS/MS	<10.0 µg/kg
Trichothecene (T-2)	LC-MS/MS	<10.0 µg/kg
Zearalenone (ZON)	LC-MS/MS	<2.0µg/kg
Fumonisin FB1	SPE with LC-MS/MS	<5.0 µg/kg
Fumonisin FB2	SPE with LC-MS/MS	<5.0 µg/kg
Sterigmatocystin	LC-MS/MS	<10.0 µg/kg
Patulin	LC-MS/MS	<10.0 µg/kg
Cyclopiazonic acid (CPA)	LC-MS/MS	<7.5 µg/kg
Citrinin	LC-MS/MS	<7.5 µg/kg
Penicillic acid	LC-MS/MS	<7.5 µg/kg

* No mycotoxins were detected; results are reported as "<" the limits of detection stated.

In addition to no CPA (or other mycotoxins) being detected in Amano's lipase from *P. camemberti*, the enzyme preparation also tested negative for antimicrobial activity. In this regard, Larsen and Knochel (1997) attributed antimicrobial activity by *P. camemberti*, which was found in 10 of 13 strains tested, to volatile compounds, including ethanol and 3-methylbutanol. Volatile compounds, if any, would be vaporized under the processing conditions in manufacture of the lipase and production of food ingredients made using the lipase.

The results of mycotoxin screening of the lipase enzyme (discussed above), in combination with the publicly available toxicological data, support Amano's conclusion that lipase from *P. camemberti* AE-LGS is not known or expected to contain toxins, antibiotics, or other substances that are not suitable for use in food. In this regard, *P. camemberti* AE-LG (from which AE-LGS was derived) has been used with no reports of safety issues for more than ten years to produce the lipase enzyme preparation as described in GRN 68. *See* FDA's "No Questions" letter dated May 29, 2001. Further, toxicological testing reported by Kondo et al. (1994) of Lipase G Amano 50 (LGK)

produced by the commercial process without the final purification and dilution steps, as discussed at pages 5-6 of GRN 68, support Amano's determination that its lipase enzyme preparation derived from *P. camemberti* is not mutagenic and presents no safety concerns when used as intended.

The no-observed-adverse-effect level (NOAEL) for LGK (the concentrated enzyme) administered by gavage to Sprague-Dawley rats in the 90-day study (discussed in detail in GRN 68) was determined to be the high dose level, 2000 mg/kg body weight. Based on the total organic solids (TOS) content of the LGK (*i.e.*, 75.76%), the NOAEL for the lipase enzyme preparation may be expressed as 1515 mg/kg body weight TOS (2000 mg/kg bw x 0.7576 = 1515 mg/kg bw). The safety margin between the NOAEL and the EDI for Amano's lipase (*i.e.*, 3.8 mg TOS/kg bw/day) is calculated to be 399-fold (1515 mg/kg bw NOAEL ÷ 3.8 mg TOS/kg bw/day = 398.7).

Safety margins greater than 100 support the conclusion that the toxicological data demonstrate the safety of the proposed uses for Amano's food production enzyme. Therefore, even with the conservative assumptions employed in estimating the daily intake of the enzyme, there are still adequate safety margins for potential dietary exposure to the enzyme when Amano's lipase derived from *P. camemberti* is used as intended in dairy processing and fats/oil processing. Further, the lack of enzyme activity in the food ingredients further diminishes the risk of any toxicity as well.

6.3 No allergenicity concerns for enzyme preparation from *Penicillium camemberti*

In contrast to most known food allergens, which are naturally present in a narrow range of foods, a wide variety of enzyme classes (and structures), are naturally present in many foods. Further, enzymes have a long history of safe use in food processing, with no indication of adverse effects or reactions. Such is the case with acylglycerol lipase. Additionally, Amano found no matches in comparing the amino acid sequence of the acylglycerol lipase to the primary sequences of known allergens based on a search of the Structural Database of Allergenic Proteins, including a search for ≥ 35% homology over a sliding "window" of 80 amino acids, and an exact match of 8 contiguous amino acids.

Regarding potential impurities, the manufacturing process for the subject lipase enzyme preparations employ standard materials that are routinely used in the enzyme industry. No target protein was detected at a maximum limit of detection of 1.4 ppm in representative batches of Lipase GS "Amano" 250G and Lipase G "Amano" 50 that were tested by enzyme-linked immunosorbent assay (ELISA) for ingredients used in production of the enzymes that contain protein derived from one of the "major food allergens" designated by the Food Allergen Labeling and Consumer Protection Act (FALCPA), *i.e.*, milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, and soybeans. Furthermore, the production media will be consumed by the microorganism as the nutrient in the fermentation process.

Additional indicators of a lack of allergenic potential for the lipase from *Penicillium camemberti* are noted as follows:

- Bindslev-Jensen *et al.* (2006) concluded that food allergy is not likely to be a concern regarding ingestion of food enzymes in general based on a study of enzymes produced by wild-type and genetically modified strains, as well as wild-type enzymes and Protein Engineered variants in 400 patients diagnosed with allergies to inhalation allergens, food allergens, bees, or wasps.

- An expert group convened by the Association of Manufacturers & Formulators of Enzyme Products (AMFEP), *i.e.*, the AMFEP Working Group on Consumer Allergy Risk from Enzyme Residues in Food, evaluated the existing scientific data and concluded that for exposure by ingestion, as opposed to exposure by inhalation, enzyme proteins are not potent allergens and sensitization to ingested enzymes is rare.

- Wüthrich (1996) published a list of enzymes that are often ingested daily over many years as digestive aids, at much higher amounts compared to enzymes present in food (up to 1 million times more), and concluded that such enzymes are not potent allergens by ingestion.

Thus, scientific data do not indicate that small amounts of enzymes in food can sensitize or induce allergic reactions in consumers. Additional considerations supporting the conclusion that allergenicity is not a concern for ingestion of enzymes in foods are outlined as follows:

- Most proteins are not food allergens and based on previous experience, the enzyme industry is not aware of any enzyme proteins used in food that are homologous to known food allergens.

- Whereas food enzymes are used in small amounts during food processing, resulting in very small amounts of the enzyme protein, if any, in the final food, we noted that a high concentration generally equals a higher risk of sensitization, whereas a low level in the final food equals a lower risk. *See e.g.*, Goodman *et al.* (2008) and the World Health Organization (WHO; 2001).

- Where proteins are denatured, as is lipase used to produce food or food ingredients in this case, the tertiary conformation of the enzyme molecule is destroyed, which is an alteration in protein conformation that is generally associated with a decrease in the antigenic reactivity in humans in the vast majority of investigated cases where denatured proteins are found to be much less immunogenic than the corresponding native proteins. *See e.g.*, Kikuchi *et al.* (2006), Nakazawa *et al.* (2005), Takai *et al.* (1997), and Valenta (2002a and 2002b).

6.4 Pariza and Johnson decision tree analysis

The enzyme preparation derived from *Penicillium camemberti* AE-LGS may be "accepted" for the intended use per an analysis using the Pariza and Johnson decision tree (Pariza and Johnson, 2001) as presented in **Figure 5** below.

Figure 5. Pariza and Johnson decision tree analysis of the lipase from *P. camemberti* AE-LGS

1. *Is the production strain genetically modified?*

    YES; *Penicillium camemberti* AE-LG (U-150) (the recipient strain) was transformed using a protoplast method by the insertion of the lipase gene (mdlA) and a marker gene (pyrG, i.e., orotidine-5'-phosphate decarboxylase) from *Penicillium camemberti* AE-LG (U-150) .(the donor strain), yielding the lipase production strain, *Penicillium camemberti* AE-LGS.

If yes, go to 2. If no, go to 6.

2. *Is the production strain modified using rDNA techniques?*

    NO; *Penicillium camemberti* AE-LGS does not contain DNA from a different species (as it is self-cloned from *P. camemberti* AE-LG).

If yes, go to 3. If no, go to 5.

5. *Is the production strain sufficiently well characterized so that one may reasonably conclude that unintended pleiotropic effects, which may result in the synthesis of toxins or other unsafe metabolites, will not arise due to the genetic modification method that was employed?*

    YES; the base production strain is non-pathogenic and non-toxigenic. Moreover, the final enzyme product is highly purified, such that the production organism is not present. *Penicillium camemberti* is well known for its long history of safe use in cheese-making where it is used to give a white felt on the surface of Coulommier and Camembert type cheese, as reported by G. Stanley in Encyclopedia of Food Sciences and Nutrition (Second Edition), 2003. Further, traditional toxicological studies have been performed that support the safety of the product.

If yes, go to 6. If no, go to 7.

6. *Is the production strain derived from a safe lineage, as previously demonstrated by repeated assessment via this evaluation procedure?*

    YES

If yes, the test article is **ACCEPTED**. *If no, go to 7.*

While the lipase enzyme preparation derived from *P. camemberti* may be considered safe (*i.e.*, ACCEPTED) by analysis stopping at step 6 under the Pariza and Johnson decision tree, we note that the organism is not pathogenic (step 7), is free of antibiotics (step 8), is free of oral toxins so known to be produced by other members of the same species

(step 9, which leads to skipping step 10), and the NOAEL for the test article in an appropriate oral study is sufficiently high to ensure safety (step 11, which results in concluding the test article is ACCEPTED).

6.5 Conclusion

Based on the documentation provided in this GRAS notification, and as discussed above, Amano has concluded that its lipase enzyme preparation derived from *Penicillium camemberti* is GRAS via scientific procedures for use as a processing aid in (1) dairy processing (*i.e.*, the production of enzyme-modified cheese) and (2) fats and oil processing (*i.e.*, the production of ingredients derived from fats and oils including, but not limited to, gamma linolenic acid (GLA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA)).

Part 7 – List of supporting data and information

7.1 References

Amano Enzyme Inc., GRN 68, Lipase from *Penicillium camembertii*, filed January 31, 2001, closed with "no questions" from FDA on May 29, 2001.

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Rao LB, Husain A (1985) Presence of cyclopiazonic acid in kodo millet (*Paspalum scrobiculatum*) causing kodua poisoning in man and its production by associated fungi. Mycopathologia 89: 177-180.

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7.2 Tables

Table 1 Comparison of TOS content of lipases derived from *P. camemberti* AE-LG and *P. camemberti* AE-LGS (self-cloned from *P. camemberti* AE-LG).

Table 2 Specifications for the lipase enzyme preparation.

7.3 Figures

Viebrock, Lauren

From: Drozen, Melvin S. <Drozen@khlaw.com>
Sent: Friday, May 29, 2020 9:56 AM
To: Viebrock, Lauren
Cc: Alsobrook, Lisa P.
Subject: RE: Acknowledgement of filing of GRAS Notice No. GRN 000908
Attachments: 2020_03_20 Filing Letter GRN 908 Transmittal.pdf

Follow Up Flag: Follow up
Flag Status: Flagged

Hi Dr. Viebrock,

We hope all is well with you.

We are just checking on the status of the review for this GRAS notice. We were hopeful and expected that because this notice is substantially the same as GRN 866 the review would go smoothly and efficiently. Do let us know if you have have any questions and we would of course appreciate a status update.

Thanks very much.

Mel Drozen.

Melvin S. Drozen
Partner
tel: +1 202.434.4222 | fax: +1 202.434.4646 | drozen@khlaw.com
1001 G Street NW, Suite 500 West | Washington, DC 20001

From: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Sent: Friday, March 20, 2020 11:08 AM
To: Drozen, Melvin S. <Drozen@khlaw.com>
Subject: Acknowledgement of filing of GRAS Notice No. GRN 000908

Dear Mr. Drozen,

Attached to this email, please find the acknowledgement letter for filing of the GRAS notice you submitted to our office, which has been designated as GRAS Notice No. GRN 000908.

Please let me know if you have any questions.

Regards,
Lauren VieBrock

Lauren VieBrock
Consumer Safety Officer/Microbiology Reviewer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
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lauren.viebrock@fda.hhs.gov

Viebrock, Lauren

From: Drozen, Melvin S. <Drozen@khlaw.com>
Sent: Tuesday, July 28, 2020 1:38 PM
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Attachments: 2020_03_20 Filing Letter GRN 908 Transmittal.pdf

Dear Dr. Viebrock,

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Dear Lauren,

Thanks for getting back to us. We look forward to hearing from you. Regards. Mel.

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Keller and Heckman LLP's Food and Drug Practice is a Chambers USA recognized Band 1 firm.

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Viebrock, Lauren

From: Drozen, Melvin S. <Drozen@khlaw.com>
Sent: Monday, September 14, 2020 10:36 AM
To: Viebrock, Lauren
Cc: Alsobrook, Lisa P.
Subject: RE: Acknowledgement of filing of GRAS Notice No. GRN 000908

Dear Dr. Viebrock,

Your August 11, 2020 email below informs us that you will be in touch regarding the status of FDA's review for GRN 908. We are touching base again for a new update because our client, Amano, has requested information from us on the status of their GRAS notice.

Thanks very much. Mel Drozen.

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Cc: Alsobrook, Lisa P. <alsobrook@khlaw.com>
Subject: RE: Acknowledgement of filing of GRAS Notice No. GRN 000908

Hi Dr. Viebrock,

We hope all is well with you.

We are just checking on the status of the review for this GRAS notice. We were hopeful and expected that because this notice is substantially the same as GRN 866 the review would go smoothly and efficiently. Do let us know if you have have any questions and we would of course appreciate a status update.

Thanks very much.

Mel Drozen.

Melvin S. Drozen
Partner
tel: +1 202.434.4222 | fax: +1 202.434.4646 | drozen@khlaw.com
1001 G Street NW, Suite 500 West | Washington, DC 20001

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From: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Sent: Friday, March 20, 2020 11:08 AM
To: Drozen, Melvin S. <Drozen@khlaw.com>
Subject: Acknowledgement of filing of GRAS Notice No. GRN 000908

Dear Mr. Drozen,

Attached to this email, please find the acknowledgement letter for filing of the GRAS notice you submitted to our office, which has been designated as GRAS Notice No. GRN 000908.

Please let me know if you have any questions.

Regards,
Lauren VieBrock

Lauren VieBrock
Consumer Safety Officer/Microbiology Reviewer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 301-796-7454
lauren.viebrock@fda.hhs.gov

Viebrock, Lauren

From: Drozen, Melvin S. <Drozen@khlaw.com>
Sent: Wednesday, September 16, 2020 1:30 PM
To: Viebrock, Lauren
Cc: Alsobrook, Lisa P.
Subject: RE: GRN 000908

Dear Lauren,

This acknowledges receipt of your email extending the review time for GRN 908.

I would be remiss if I did not express our disappointment, on behalf of Amano, for the extension of the review time here. GRN 908 replaced GRN 866, which itself had undergone reasonable review by OFAS when we agreed, reluctantly, to withdraw the notice. We understand that there are many GRN's under review, but under the circumstances here, we had hoped and expected this review to go more quickly.

Best regards,

Mel.

From: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Sent: Wednesday, September 16, 2020 12:57 PM
To: Drozen, Melvin S. <Drozen@khlaw.com>
Cc: Alsobrook, Lisa P. <alsobrook@khlaw.com>
Subject: GRN 000908

Dear Mr. Drozen,

This email is to inform you that, in accordance with 21 CFR 170.265 (b)(2), FDA is extending the normal 180 day review timeframe by 90 days. The original 180 day date for GRN 000908 is 9/16/20.

Regards,

Lauren

Lauren VieBrock
Regulatory Review Scientist/Microbiology Reviewer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 301-796-7454
lauren.viebrock@fda.hhs.gov

Viebrock, Lauren

From: Drozen, Melvin S. <Drozen@khlaw.com>
Sent: Wednesday, September 16, 2020 1:59 PM
To: Viebrock, Lauren
Cc: Alsobrook, Lisa P.
Subject: RE: Acknowledgement of filing of GRAS Notice No. GRN 000908

Hello Lauren,

Just saw this and had already responded to the 90 day extension email.

We're glad the review is moving forward, but we are disappointed that this one has taken quite this long since this GRN replaces GRN 866 which had also been under review for some time, and contains substantially the same information. Hopefully your review can be completed favorably soon.

Thanks and regards,

Mel.

From: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Sent: Wednesday, September 16, 2020 12:56 PM
To: Drozen, Melvin S. <Drozen@khlaw.com>
Cc: Alsobrook, Lisa P. <alsobrook@khlaw.com>
Subject: RE: Acknowledgement of filing of GRAS Notice No. GRN 000908

Dear Mr. Drozen,

Thank you for your email. I apologize again for the delay in my response. Our review is moving forward on GRN 908, but I do not have any more specific information on a timeline at this point. We will be extending our review by 90 days and I will be sending an email with that information. Please let me know if you have any additional questions.

Best,
Lauren

From: Drozen, Melvin S. <Drozen@khlaw.com>
Sent: Monday, September 14, 2020 10:36 AM
To: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Cc: Alsobrook, Lisa P. <alsobrook@khlaw.com>
Subject: RE: Acknowledgement of filing of GRAS Notice No. GRN 000908

Dear Dr. Viebrock,

Your August 11, 2020 email below informs us that you will be in touch regarding the status of FDA's review for GRN 908. We are touching base again for a new update because our client, Amano, has requested information from us on the status of their GRAS notice.

Thanks very much. Mel Drozen.

From: Drozen, Melvin S. <Drozen@khlaw.com>
Sent: Tuesday, August 11, 2020 3:15 PM
To: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Cc: Alsobrook, Lisa P. <alsobrook@khlaw.com>
Subject: RE: Acknowledgement of filing of GRAS Notice No. GRN 000908

Dear Lauren,

Thanks for getting back to us. We look forward to hearing from you. Regards. Mel.

From: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Sent: Tuesday, August 11, 2020 1:44 PM
To: Drozen, Melvin S. <Drozen@khlaw.com>
Cc: Alsobrook, Lisa P. <alsobrook@khlaw.com>
Subject: RE: Acknowledgement of filing of GRAS Notice No. GRN 000908

Dear Mr. Drozen,

Thank you for your email. I apologize for the delay in my response. I will be in touch with you in more detail in the coming days regarding the status of our review. Thank you.

Regards,
Lauren

From: Drozen, Melvin S. <Drozen@khlaw.com>
Sent: Tuesday, July 28, 2020 1:38 PM
To: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Cc: Alsobrook, Lisa P. <alsobrook@khlaw.com>
Subject: Acknowledgement of filing of GRAS Notice No. GRN 000908

Dear Dr. Viebrock,

We are following up on our email below and hoping you can update us on the status of the review for GRN 908. Thanks very much. Mel Drozen.

Melvin S. Drozen
Partner
tel: +1 202.434.4222 | fax: +1 202.434.4646 | drozen@khlaw.com
1001 G Street NW, Suite 500 West | Washington, DC 20001

From: Drozen, Melvin S.
Sent: Friday, May 29, 2020 9:56 AM
To: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Cc: Alsobrook, Lisa P. <alsobrook@khlaw.com>
Subject: RE: Acknowledgement of filing of GRAS Notice No. GRN 000908

Hi Dr. Viebrock,

We hope all is well with you.

We are just checking on the status of the review for this GRAS notice. We were hopeful and expected that because this notice is substantially the same as GRN 866 the review would go smoothly and efficiently. Do let us know if you have have any questions and we would of course appreciate a status update.

Thanks very much.

Mel Drozen.

Melvin S. Drozen
Partner
tel: +1 202.434.4222 | fax: +1 202.434.4646 | drozen@khlaw.com
1001 G Street NW, Suite 500 West | Washington, DC 20001

From: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Sent: Friday, March 20, 2020 11:08 AM
To: Drozen, Melvin S. <Drozen@khlaw.com>
Subject: Acknowledgement of filing of GRAS Notice No. GRN 000908

Dear Mr. Drozen,

Attached to this email, please find the acknowledgement letter for filing of the GRAS notice you submitted to our office, which has been designated as GRAS Notice No. GRN 000908.

Please let me know if you have any questions.

Regards,

Lauren VieBrock
Consumer Safety Officer/Microbiology Reviewer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 301-796-7454
lauren.viebrock@fda.hhs.gov

Viebrock, Lauren

From: Drozen, Melvin S. <Drozen@khlaw.com>
Sent: Thursday, September 17, 2020 9:55 AM
To: Viebrock, Lauren
Cc: Alsobrook, Lisa P.
Subject: RE: Acknowledgement of filing of GRAS Notice No. GRN 000908

Hi Lauren,

Thanks so much for getting back to us. We are sure you will do what you can. Best. Mel.

Melvin S. Drozen
Partner
tel: +1 202.434.4222 | fax: +1 202.434.4646 | drozen@khlaw.com
1001 G Street NW, Suite 500 West | Washington, DC 20001

From: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Sent: Thursday, September 17, 2020 9:53 AM
To: Drozen, Melvin S. <Drozen@khlaw.com>
Cc: Alsobrook, Lisa P. <alsobrook@khlaw.com>
Subject: RE: Acknowledgement of filing of GRAS Notice No. GRN 000908

Hi Mel,

Thank you for your emails. I understand the frustration and we will continue to move forward with the review and response as quickly as we can.

Best,
Lauren

From: Drozen, Melvin S. <Drozen@khlaw.com>
Sent: Wednesday, September 16, 2020 1:59 PM
To: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Cc: Alsobrook, Lisa P. <alsobrook@khlaw.com>
Subject: RE: Acknowledgement of filing of GRAS Notice No. GRN 000908

Hello Lauren,

Just saw this and had already responded to the 90 day extension email.

We're glad the review is moving forward, but we are disappointed that this one has taken quite this long since this GRN replaces GRN 866 which had also been under review for some time, and contains substantially the same information. Hopefully your review can be completed favorably soon.

Thanks and regards,

Mel.

From: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Sent: Wednesday, September 16, 2020 12:56 PM
To: Drozen, Melvin S. <Drozen@khlaw.com>
Cc: Alsobrook, Lisa P. <alsobrook@khlaw.com>
Subject: RE: Acknowledgement of filing of GRAS Notice No. GRN 000908

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Thank you for your email. I apologize again for the delay in my response. Our review is moving forward on GRN 908, but I do not have any more specific information on a timeline at this point. We will be extending our review by 90 days and I will be sending an email with that information. Please let me know if you have any additional questions.

Best,
Lauren

From: Drozen, Melvin S. <Drozen@khlaw.com>
Sent: Monday, September 14, 2020 10:36 AM
To: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Cc: Alsobrook, Lisa P. <alsobrook@khlaw.com>
Subject: RE: Acknowledgement of filing of GRAS Notice No. GRN 000908

Dear Dr. Viebrock,

Your August 11, 2020 email below informs us that you will be in touch regarding the status of FDA's review for GRN 908. We are touching base again for a new update because our client, Amano, has requested information from us on the status of their GRAS notice.

Thanks very much. Mel Drozen.

From: Drozen, Melvin S. <Drozen@khlaw.com>
Sent: Tuesday, August 11, 2020 3:15 PM
To: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Cc: Alsobrook, Lisa P. <alsobrook@khlaw.com>
Subject: RE: Acknowledgement of filing of GRAS Notice No. GRN 000908

Dear Lauren,

Thanks for getting back to us. We look forward to hearing from you. Regards. Mel.

From: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Sent: Tuesday, August 11, 2020 1:44 PM
To: Drozen, Melvin S. <Drozen@khlaw.com>
Cc: Alsobrook, Lisa P. <alsobrook@khlaw.com>
Subject: RE: Acknowledgement of filing of GRAS Notice No. GRN 000908

Dear Mr. Drozen,

Thank you for your email. I apologize for the delay in my response. I will be in touch with you in more detail in the coming days regarding the status of our review. Thank you.

Regards,
Lauren

From: Drozen, Melvin S. <Drozen@khlaw.com>
Sent: Tuesday, July 28, 2020 1:38 PM
To: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Cc: Alsobrook, Lisa P. <alsobrook@khlaw.com>
Subject: Acknowledgement of filing of GRAS Notice No. GRN 000908

Dear Dr. Viebrock,

We are following up on our email below and hoping you can update us on the status of the review for GRN 908. Thanks very much. Mel Drozen.

Melvin S. Drozen
Partner
tel: +1 202.434.4222 | fax: +1 202.434.4646 | drozen@khlaw.com
1001 G Street NW, Suite 500 West | Washington, DC 20001

From: Drozen, Melvin S.
Sent: Friday, May 29, 2020 9:56 AM
To: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Cc: Alsobrook, Lisa P. <alsobrook@khlaw.com>
Subject: RE: Acknowledgement of filing of GRAS Notice No. GRN 000908

Hi Dr. Viebrock,

We hope all is well with you.

We are just checking on the status of the review for this GRAS notice. We were hopeful and expected that because this notice is substantially the same as GRN 866 the review would go smoothly and efficiently. Do let us know if you have have any questions and we would of course appreciate a status update.

Thanks very much.

Mel Drozen.

Melvin S. Drozen
Partner
tel: +1 202.434.4222 | fax: +1 202.434.4646 | drozen@khlaw.com
1001 G Street NW, Suite 500 West | Washington, DC 20001

From: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Sent: Friday, March 20, 2020 11:08 AM
To: Drozen, Melvin S. <Drozen@khlaw.com>
Subject: Acknowledgement of filing of GRAS Notice No. GRN 000908

Dear Mr. Drozen,

Attached to this email, please find the acknowledgement letter for filing of the GRAS notice you submitted to our office, which has been designated as GRAS Notice No. GRN 000908.

Please let me know if you have any questions.

Regards,
Lauren VieBrock

Lauren VieBrock
Consumer Safety Officer/Microbiology Reviewer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 301-796-7454
lauren.viebrock@fda.hhs.gov

Viebrock, Lauren

From: Drozen, Melvin S. <Drozen@khlaw.com>
Sent: Thursday, November 12, 2020 2:38 PM
To: Viebrock, Lauren
Cc: Alsobrook, Lisa P.
Subject: RE: GRN 908 Questions
Attachments: 2020_11_12 GRN 908 Questions for the notifier.pdf

Dear Ms. Viebrock,

Thank you for your email. We will review and get back to you. Regards. Mel Drozen.

Melvin S. Drozen
Partner
tel: +1 202.434.4222 | fax: +1 202.434.4646 | drozen@khlaw.com
1001 G Street NW, Suite 500 West | Washington, DC 20001

From: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Sent: Thursday, November 12, 2020 1:20 PM
To: Drozen, Melvin S. <Drozen@khlaw.com>
Subject: GRN 908 Questions

Dear Mr. Drozen,

During our review of GRAS Notice No. 000908, we noted questions that need to be addressed and are attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Regards,
Lauren

Lauren VieBrock
Regulatory Review Scientist/Microbiology Reviewer
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 301-796-7454
lauren.viebrock@fda.hhs.gov

Viebrock, Lauren

From: Drozen, Melvin S. <Drozen@khlaw.com>
Sent: Monday, November 30, 2020 9:22 AM
To: Viebrock, Lauren
Cc: Alsobrook, Lisa P.
Subject: RE: GRN 908 Questions
Attachments: 2020_11_12 GRN 908 Questions for the notifier.pdf

Dear Lauren,

We hope you are well and had a nice Thanksgiving holiday. The holiday week has delayed our ability to provide a response to your questions but we expect to have a response to you by the end of the week. Best, Mel Drozen.

From: Drozen, Melvin S.
Sent: Thursday, November 12, 2020 2:38 PM
To: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Cc: Alsobrook, Lisa P. <alsobrook@khlaw.com>
Subject: RE: GRN 908 Questions

Dear Ms. Viebrock,

Thank you for your email. We will review and get back to you. Regards. Mel Drozen.

Melvin S. Drozen
Partner
tel: +1 202.434.4222 | fax: +1 202.434.4646 | drozen@khlaw.com
1001 G Street NW, Suite 500 West | Washington, DC 20001

From: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Sent: Thursday, November 12, 2020 1:20 PM
To: Drozen, Melvin S. <Drozen@khlaw.com>
Subject: GRN 908 Questions

Dear Mr. Drozen,

During our review of GRAS Notice No. 000908, we noted questions that need to be addressed and are attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Regards,
Lauren

Lauren VieBrock
Regulatory Review Scientist/Microbiology Reviewer
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 301-796-7454
lauren.viebrock@fda.hhs.gov

Viebrock, Lauren

From: Drozen, Melvin S. <Drozen@khlaw.com>
Sent: Thursday, December 03, 2020 10:09 AM
To: Viebrock, Lauren
Cc: Alsobrook, Lisa P.
Subject: GRN 908--Response to FDA Questions
Attachments: 2020_11_12 GRN 908 Questions for the notifier.pdf; GRN 908 (response letter).pdf

Dear Lauren,

Please find the attached "GRN 908 (response letter)," which we send on behalf of our client, Amano Enzyme, Inc. (Amano), to address the questions posed by the Food and Drug Administration (FDA), attached to your November 12, 2020 email below, regarding the Generally Recognized as Safe (GRAS) notice that we submitted on January 14, 2020 to cover the intended uses of a lipase enzyme preparation derived from *Penicillium camemberti*.

We look forward to the FDA's continued review of GRN 908.

Sincerely,

Mel Drozen and Lisa Alsobrook.

From: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Sent: Thursday, November 12, 2020 1:20 PM
To: Drozen, Melvin S. <Drozen@khlaw.com>
Subject: GRN 908 Questions

Dear Mr. Drozen,

During our review of GRAS Notice No. 000908, we noted questions that need to be addressed and are attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Regards,
Lauren

Lauren VieBrock
Regulatory Review Scientist/Microbiology Reviewer
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
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lauren.viebrock@fda.hhs.gov

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Writer's Direct Access
Melvin S. Drozen
(202) 434-4222
drozen@khlaw.com

December 3, 2020

Via Electronic Mail

Lauren VieBrock, Ph.D.
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Campus Drive
College Park, MD 20740

Re: GRN

Dear Dr. VieBrock:

We are writing on behalf of our client, Amano Enzyme, Inc. (Amano), to address the questions posed by the Food and Drug Administration (FDA) that are attached to your November 12, 2020 email regarding the Generally Recognized as Safe (GRAS) notice that we submitted on January 14, 2020 to cover the intended uses of a lipase enzyme preparation derived from *Penicillium camemberti*. The questions that FDA has recently raised are listed below and followed by our responses.

Question 1. Please clarify whether the integration of the introduced DNA was confirmed and specify the method used.

Response

As noted in GRN 908 (page 2), the production organism was derived by self-cloning from *P. camemberti* U-150, a non-genetically modified (GM) strain, designated internally by Amano as strain AE-LG. The lipase derived from strain AE-LG is described in Amano's GRN 68. The new, GM strain is designated internally by Amano as AE-LGS. The genetic modification is described in GRN 908 (page 11). Specifically, *P. camemberti* AE-LGS contains two genes from *P. camemberti* AE-LG: (1) native lipase gene, mdlA, and (2) marker gene, pyrG (orotidine-5'-phosphate decarboxylase) for non-toxic nutritional selection of transformed cells with amplified mdlA genes. Using a pyrG-deficient strain derived from UV treatment of *P. camemberti* AE-LG as the host, transformation was conducted by the protoplast method to achieve the target transformant. The DNA fragments mdlA and pyrG were incorporated into the host genome through non-homologous recombination. Transformants were grown on a plate

spiked with brilliant green and monoolein, and primary screening was conducted based on the Halo size. Strains with high lipase productivity were selected.

Additionally, early in the development phase, Amano used a Polymerase Chain Reaction (PCR) assay of the transformant spores to confirm integration of the introduced DNA using primers specific to mdlA/pyrG.

Question 2. Please clarify whether the stability of the introduced DNA has been confirmed.

Response

The genetic stability of *P. chrysogenum*, a congenetic species of *P. camemberti*, is well known (Renno DV et al., 1990).[1] Consistent expression of the lipase enzyme for numerous generations indicates that the mdlA gene has been stably inserted into the host genome.

Question 3. Please confirm that the final production strain does not contain any functional or transferable antibiotic resistance genes.

Response

The final production strain does not contain any functional or transferable antibiotic resistance genes.

The production strain was self-cloned from *P. camemberti*, and therefore it does not contain DNA from a different species. Further, no antibiotic resistance genes were used in the self-cloning process. Specifically, *Penicillium camemberti* AE-LGS was made by adding native lipase gene, mdlA, to *P. camemberti* AE-LG as the donor organism and using pyrG (orotidine-5'-phosphate decarboxylase) as the marker gene.

Question 4. Please clarify whether the manufacturing specifications include the absence of the production strain.

Response

As noted in GRN 908 (page 22), the final enzyme product is highly purified, such that the production organism is not present. The production strain is removed by separation of the biomass from the spent broth (which contains the enzyme) in the first filtration step following fermentation, as described in GRN 68 (which was incorporated by reference in GRN 908) and is

[1] Renno.V. D and Saunders. G. (1990) The genetic stability of *Penicillium chrysoyenum* transformants in a fermentor, Appl Microbiol Biotechnol 34:364-367.

further clarified in response to FDA's Question 6(a) below. Subsequent filtration steps at the end of the recovery process result in a concentrated enzyme solution free of the production strain and insoluble substances.

Question 5. In Part 1.3 of the notice, under the "Dairy processing" subheading, it is stated that "lipase is added to emulsified cheese homogenates (the raw material), along with animal or microbial enzymes, at levels ranging from 25 to 2,522 mg TOS/kg raw material during the incubation stage in making enzyme modified cheese (EMC). Please clarify:

(a) The point at which the enzyme is added—is it added to raw or pasteurized milk intended for cheese production (as suggested by concomitant addition of other enzymes) or is it added to prepared cheese (as suggested by the term "emulsified cheese homogenates)?

(b) The reason for broad use levels of the enzyme in enzyme-modified cheese and fats/oil. Is it based on the monoacylglycerol content of the oil or a specific end point?

Response

The point at which the enzyme is added in "dairy processing," where the enzyme is used to enhance the flavor, depends upon the raw materials. The enzyme may be added directly to the milk in the initial stage of cheese production, or in different "dairy processing" operations, the enzyme may be added to the cheese or cheese curd at various stages, such as crushing, mixing/kneading, or other processing steps.

The addition rate of the enzyme to milk is relatively low because the ratio of the substrate to the total weight is low. When the enzyme is added to cheese or cheese curd, however, the substrate content is higher because water, such as from whey, has been removed and, so the enzyme addition rate also is higher. The range of 25 to 2,522 mg TOS/kg raw material reflects the range of manufacturing processes for enzyme modified cheese (EMC), including processes where the enzyme is added to milk, at lower levels, and processes where the enzyme is added to cheese or cheese curd, at higher levels.

It is difficult to quantify the effect of the enzyme for "dairy processing," such as producing the desired flavor, umami, and maturity profile for a particular finished food made with EMC, in a way that can be associated with analytical data on the fatty acid composition of the raw material. In practice, sensory testing is used to determine the appropriate use level for each specific application and the range in use levels tends to be wide.

The purpose of the enzyme in "fats and oils processing" is to obtain high yields of fats and oils containing a high amount of triglycerides by esterifying partial glycerides, which are impurities contained in fats and oils, without changing the triglyceride itself. Thus, the fatty acid

composition of the raw material in this case can provide a guideline for the use level of the enzyme, and the range in use levels for different raw materials in "fats and oils processing" is more narrow than for "dairy processing" applications.

Question 6. As currently written, Section 2.2 of the notice (page 15) includes reference to a previous notice and a statement that "A detailed description of the manufacturing process, which was provided in GRN 68 and is incorporated herein, reflects the current process with the exception of minor changes made to improve process efficiency. *See* GRN 68 at Appendix I, located at page 13 of GRN 68." The actual method of enzyme production and subsequent purification is not described in GRN 908. We request that you provide:

(a) A brief summary of the method of manufacture that incorporates modifications (e.g., optimization of the filtration process) to the manufacturing process.

(b) A statement that all food contact materials are safe and suitable for their intended use, including a citation to an effective food contact notification (FCN) or food contact regulation for the use of that material. While we do see a statement regarding "raw materials", this statement does not include a citation to an FCN or regulation, and it is unclear if the term "raw materials" is broadly used or refers specifically to foods treated with the enzyme.

(c) Describe food grade media components, identifying potential sources of allergenic proteins. While you note in Part 6.3 (p. 20) that none are present, it is not clear if this statement reflects that they were not used in the fermentation process or if they were effectively removed by the method of manufacture. If the latter, please include this information in the summary of the method of manufacture, indicating which step(s) in the purification process ensure removal of allergens.

Response

The production strain secretes the enzyme into the culture medium during a controlled fermentation of the *P. camemberti* strain with conditions specified for time, temperature, and rate of rotation. Conditions specified for temperature (26ºC) and rotation (125 rpm) are the same as described in GRN 68. Whereas GRN 68 states that the enzyme is harvested after approximately 66 hours of fermentation, however, the lipase is now harvested earlier (at 40 to 50 hours) to avoid clogging of the filter that is used to separate the biomass from the medium (which contains the enzyme). Amano separates the enzyme from the cells by a sequence of filtration steps (using press filters and ultrafilters for a 6000 molecular weight cut), followed by heat treatment and pH adjustment (to pH 6.4 to 6.6), then additional filtration (press filter and ceramic filter). The resulting fluid is then precipitated with the addition of cold ethanol. The resulting precipitate is centrifuged, dried, crushed, and blended with a diluent to the desired enzyme activity for the

finished product. To dilute the enzyme preparation to its final activity, Amano uses maltodextrin, which FDA affirmed as GRAS in 21 C.F.R. § 184.1444. The final enzyme preparation is sieved through a 32-mesh screen.

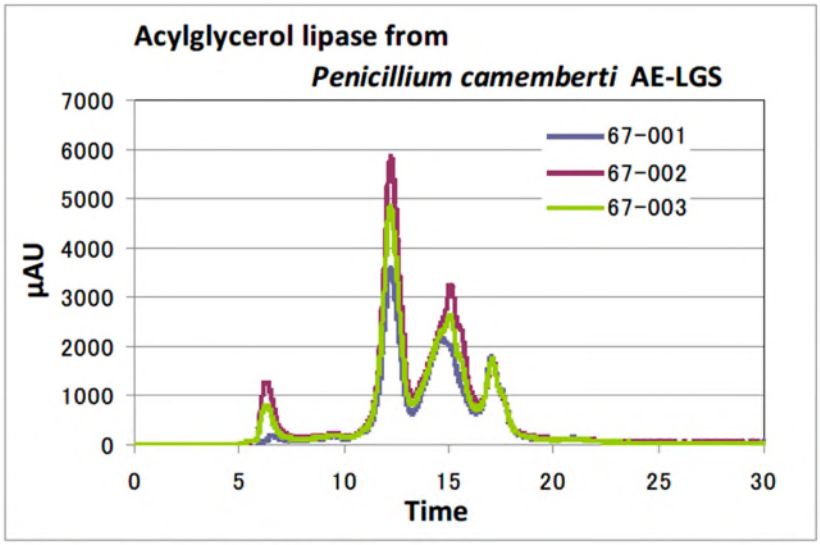
Again, all manufacturing steps, which are summarized in-brief above, have been described in GRN 68. For the course-filtering step (where the biomass is removed), however, and the two press filter steps, Amano has now specified diatomaceous earth for filtering, whereas no filtering medium was specified in GRN 68. The raw materials used in fermentation (*e.g.*, carbon, nitrogen and vitamin sources, salts and minerals, antifoam agents, pH adjustment aids) and in enzyme recovery, *i.e.*, processing aids (*e.g.*, salts and minerals, stabilizer, pH adjustment aids) and food contact materials (*e.g.*, filtering aids), are of food grade quality and are used in accordance with an appropriate food regulatory clearance or have been determined to be GRAS for their respective uses, or in the case of antifoam agents used in fermentation, the substances are used in accordance with a September 11, 2003 letter from FDA in response to a request from the Enzyme Technical Association (ETA) for a review of certain defoaming and flocculating agents in the manufacture of enzyme preparations used in food.[2] All raw materials used in fermentation and recovery of the enzyme are safe and suitable for such use.

The raw materials used in the fermentation step include potable water and the nutrients necessary for growth of the production strain. No ingredient used in the fermentation media, aside from a soybean derivative, is derived from a major food allergen. Post-fermentation processing includes filtration to remove cell biomass and large molecules (including soy protein) from the fermentate. Analysis of three non-nonconsecutive batches of the enzyme by a commercially available enzyme-linked immunosorbent assay (ELISA) test kit confirms that soy protein was not detected at the limit of detection (LOD) of 1 ppm.

The protein profile of three samples of the lipase derived from the self-cloned strain of *P. camemberti* (designated internally by Amano as AE-LGS) is illustrated by chromatographic separation in Figure 1. below:

[2] The letter to ETA was located on FDA's website at http://wayback.archive-it.org/7993/20171031044546/https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/UCM313038.pdf. *See* page 128–132.

Figure 1. Protein profile of the lipase derived from P. camemberti AE-LGS.



- Column: TSK-Gel 3000SW (Tosoh)
- Solvent: 0.3M NaCl in 0.05M Phosphate 1K-2Na buffer (pH 7.0)
- Flow rate: 1.0 mL/min
- Detection: 280nm
- Temperature: 25°C
- Detection time: 60 min
- Injection: 0.5% (w/v) Sample, 50 μL

This is the same protein profile as the lipase from the non-GM strain (designated internally by Amano as strain AE-LG) which is covered by GRN 68.

Question 7. Regarding specifications provided in Section 2.3 (p. 16) and results listed in Figure 4 (p. 23), please provide citation to the methods used to assess antimicrobial activity and mycotoxins. If these are not official methods (e.g., AOAC or other), please provide a statement that the methods have been validated and are fit for purpose.

Response

"Determination of antibiotic activity" was assessed by the method described in the Compendium of Food Additive Specifications, vol. 2, Joint FAO/WHO Expert Committee on Food Additives (JECFA), Food and Agriculture Organization of the United Nations, Rome, 1992.

The detection methods for the mycotoxins listed in GRN 908, Table 4 (page 19), *i.e.*, High-Performance Liquid Chromatography with Fluorescence Detector (HPLC/FD) for

aflatoxins and ochratoxins; a LC–mass spectrometry (MS)/MS system for deoxynivalenol, fusarium, trichothecene, zearalenone, sterigmatocystin, patulin, cyclopiazonic acid, citrinin, and penicillic acid; and an online solid phase extraction-liquid chromatography-tandem mass spectrometry (SPE-LC-MS/MS) system for fumonisins, are based on Association of Official Analytical Chemists (AOAC) and other food industry approved methods that have been deemed fit for the purpose.

Question 8. In Part 3, dietary exposure (p. 17), you provide an exposure estimate based on the following assumptions:

(a) the enzyme use level differs for the two applications: 2522 mg TOS/kg enzyme-modified cheese; 252 mg/kg edible fats and oils

(b) The enzyme modified cheese and fats/oils are used at the following stated levels in final food categories:

i) enzyme modified cheese is used in soups, snack foods, and processed cheese at 2% by weight.

ii) enzyme-modified fats and oils are used in bread, pastry, and confections at 30% by weight.

(c) The enzyme is present at levels of 76 mg TOS/kg food in the diet and that 3 kg food and beverage are consumed per day.

i) We request that you clarify the following:

(1) Whether the use levels "ratio RM/final food" of the enzyme-modified cheese and fat/oil are considered to be typical or maximum levels.

(2) Any beverage uses of the enzyme-modified fat/oil.

(3) If the uses of the GRN 908 enzyme in fat/oils replace the current uses for lipase from *P. camemberti* or if described in GRN 68.

As clarified in response to FDA's Question 5 above, the lipase enzyme preparation is used at levels ranging from 25 to 2,522 mg TOS/kg raw material (*i.e.*, milk, cheese, or cheese curd) and 50 to 252 mg TOS/kg raw material (*i.e.*, fat or oil) in the dairy processing and fat/oil processing applications, respectively. For the dairy processing applications, although the initial addition rate of the enzyme is lower when the enzyme is added to milk, as opposed to when the enzyme is added to cheese or cheese curd, the level of the enzyme in the finished EMC is expected to be roughly similar after considering that further water removal occurs after the addition of the enzyme when making EMC starting with the addition of the enzyme to milk. Thus, the use level of 2,522 mg TOS/kg raw material, which is the maximum addition rate of the enzyme when added to cheese (*i.e.*, EMC) as the raw material, is considered to be the maximum use level of the enzyme in the dairy processing application. Thus, the values of 2522 and 252, for dairy processing and fats/oil processing, respectively, in the columns labeled "Maximum

Recommended Use Level (mg TOS/kg RM)" in Table 4 of GRN 908 (Part 3) represent the maximum levels at which the enzyme may be present in EMC and a fat/oil ingredient, respectively. The final foods that may contain as an ingredient the EMC made with Amano's enzyme (i.e., soup, snack foods, processed cheese) or the fat/oil made with Amano's enzyme (*i.e.*, bread, pastry, and confections) are the "raw material" (RM) for purposes of Table 4. Thus, the values of 0.02 and 0.3 for dairy processing and fats/oil processing, respectively, in the column labeled "Ratio RM/Final Food" reflect that EMC (containing the enzyme at a maximum level of 2,522 mg TOS/kg EMC) may be used as an ingredient in a finished food at a maximum level of 2%, and a fat/oil (containing the enzyme at a maximum level of 252 mg TOS/kg fat/oil) may be used as an ingredient in a finished food at a maximum level of 30%.

Neither EMC, nor fat/oil made using the enzyme are expected to be used as ingredients in a beverage.

The use of the enzyme in fats/oil processing to produce food ingredients, as described in GRN 908, does not replace the use of the lipase from *P. camemberti* that is described in GRN 68. The GRAS conclusion for use of the enzyme from the non-GM strain (described in GRN 68) applies as well to the use of the enzyme from the GM strain because the lipase derived from either production organism is the same (it is only the yield that is increased in the case of production using the self-cloned strain). In this regard, while the maximum potential dietary exposure to the enzyme from the earlier application, described in GRN 68, was calculated to be 0.017 mg/kg bw/day, and it is assumed that fatty acid supplements containing a fat/oil made using the enzyme (as described in GRN 68) could be consumed by persons who also consume foods containing an EMC or fat/oil ingredient made using the enzyme (as described in GRN 908), any amount of lipase from the fatty acids supplement application that is described in GRN 68, would be subsumed within the Estimated Daily Intake (EDI) for the enzyme (*i.e.*, 3.8 mg TOS/kg bw/day) that was calculated very conservatively in GRN 908. Accordingly, Amano has concluded that lipase derived from *P. camemberti* as described in GRN 908 is GRAS for processing fats or oils for use as fatty acid supplements.

Question 9. In addition to dextrin (carrier, detailed in Part 2 p. 10), please indicate any other diluents, stabilizers, preservatives and other substances (e.g., for pH adjustment) that are added to the enzyme preparation.

Maltodextrin, rather than dextrin, is currently added as a diluent (carrier) to the enzyme preparation. Maltodextrin is affirmed as GRAS at 21 C.F.R. § 184.1444 for use in food with no limitation other than current good manufacturing practice. No preservative or other ingredients are added to the enzyme preparation. Other substances noted in FDA's question above are raw materials used in the fermentation and recovery steps (discussed in response to Question 6 above). These are processing aids used in Amano's confidential manufacturing process and are not part of the formulation of the enzyme preparation. Maltodextrin (an ingredient in the enzyme preparation) and processing aids used in producing the enzyme are food grade quality.

* * *

We hope and trust that the information above responds fully to FDA's questions regarding Amano's GRN 908. We look forward to the Agency's continued review of the Notice and we would be happy to provide you with any further information you may need.

Sincerely,

Melvin S. Drozen

Viebrock, Lauren

From: Drozen, Melvin S. <Drozen@khlaw.com>
Sent: Tuesday, January 05, 2021 10:47 AM
To: Viebrock, Lauren
Cc: Alsobrook, Lisa P.
Subject: [WARNING : MESSAGE ENCRYPTED] GRN 908--Response to FDA Questions
Attachments: 2020_!2_08 GRN 908 Questions 2.pdf; Response to FDA's 12-8-2020 request (letter).pdf; Assay method for Lipase activity (LV method).pdf; Yamaguchi (1991).pdf

Follow Up Flag: Follow up
Flag Status: Completed

Dear Lauren,

Happy New Year. We hope you had a pleasant and safe holiday season.

Please find the attached "Response to FDA's 12-8-2020 request (letter)," which we send on behalf of our client, Amano Enzyme Inc. (Amano), to address the questions posed by the Food and Drug Administration (FDA), attached to your December 8, 2020 email below, regarding the Generally Recognized as Safe (GRAS) notice that we submitted on January 14, 2020 to cover the intended uses of a lipase enzyme preparation derived from *Penicillium camemberti*. References discussed in our response letter are also attached: (1) Yamaguchi (1991), and (2) Assay method for lipase activity (Confidential internal method).

We look forward to the FDA's continued review of GRN 908.

Sincerely,

Mel and Lisa.

Melvin S. Drozen
Partner
tel: +1 202.434.4222 | fax: +1 202.434.4646 | drozen@khlaw.com
1001 G Street NW, Suite 500 West | Washington, DC 20001

From: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Sent: Tuesday, December 8, 2020 1:14 PM
To: Drozen, Melvin S. <Drozen@khlaw.com>

Cc: Alsobrook, Lisa P. <alsobrook@khlaw.com>
Subject: RE: GRN 908--Response to FDA Questions

Dear Mr. Drozen,

Thank you for the information you submitted in response to our questions for GRN 000908. We have an additional questions to be addressed that are attached to this email.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Regards,
Lauren

Lauren VieBrock
Regulatory Review Scientist/Microbiology Reviewer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 301-796-7454
lauren.viebrock@fda.hhs.gov

From: Drozen, Melvin S. <Drozen@khlaw.com>
Sent: Thursday, December 03, 2020 10:09 AM
To: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Cc: Alsobrook, Lisa P. <alsobrook@khlaw.com>
Subject: GRN 908--Response to FDA Questions

Dear Lauren,

Please find the attached "GRN 908 (response letter)," which we send on behalf of our client, Amano Enzyme, Inc. (Amano), to address the questions posed by the Food and Drug Administration (FDA), attached to your November 12, 2020 email below, regarding the Generally Recognized as Safe (GRAS) notice that we submitted on January 14, 2020 to cover the intended uses of a lipase enzyme preparation derived from *Penicillium camemberti*.

We look forward to the FDA's continued review of GRN 908.

Sincerely,

Mel Drozen and Lisa Alsobrook.

From: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Sent: Thursday, November 12, 2020 1:20 PM
To: Drozen, Melvin S. <Drozen@khlaw.com>
Subject: GRN 908 Questions

Dear Mr. Drozen,

During our review of GRAS Notice No. 000908, we noted questions that need to be addressed and are attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Regards,
Lauren

Lauren VieBrock
Regulatory Review Scientist/Microbiology Reviewer
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Writer's Direct Access
Melvin S. Drozen
(202) 434-4222
drozen@khlaw.com

January 5, 2021

Via Electronic Mail

Lauren VieBrock, Ph.D.
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Campus Drive
College Park, MD 20740

Re: Response to FDA's December 8, 2020 Email with Additional Questions Regarding Amano Enzyme Inc.'s GRN 908

Dear Dr. VieBrock:

We are writing on behalf of our client, Amano Enzyme Inc. (Amano), to address the questions posed by the Food and Drug Administration (FDA) that are attached to your December 8, 2020 email regarding the Generally Recognized as Safe (GRAS) notice that we submitted on January 14, 2020 to cover the intended uses of a lipase enzyme preparation derived from *Penicillium camemberti*. We provided clarification on December 3, 2020 in response to previous questions posed by FDA, in a November 12, 2020 email. The questions that FDA has most recently raised are listed below and followed by our responses.

Question 1. On pg. 17, you state that a literature search was conducted on January 3, 2020 to assess health concerns associated with P. camemberti. Yet on pg. 18, you state: "No adverse toxicological data were located in an updated review of the toxicological literature conducted on August 23, 2018, aside from reports … which indicate that cyclopiazonic acid (CPA) is a mycotoxin of potential concern for some strains of Penicillium camemberti under certain environmental conditions." Please confirm that an updated literature search relevant to safety assessment included both the *P. camemberti* as well as lipase enzyme preparation itself.

Response:

An updated literature search, conducted on January 3, 2020, encompassed both the production organism, *P. camemberti*, as well as the lipase enzyme preparation itself, and revealed no new, relevant toxicological data published subsequent to the initial search that was

conducted on August 23, 2018 as we worked on the first iteration of this GRAS notice, *i.e.*, GRN 866 (submitted May 30, 2019, filed by FDA on August 1, 2019, and withdrawn January 13, 2020 to confirm with FDA's administrative requirements).

Question 2. 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. The enzyme, known by the systematic name glycerolester acylhydrolase, hydrolyzes glycerol monoesters of long-chain fatty acids. In the notice, you indicate that the lipase from P. camemberti hydrolyzes mono- and diglycerides. From the EC number indicated (EC No. 3.1.1.23), we can only confirm monoacylglycerol lipase activity. Please clarify:

a. -Is the diacylglycerol activity secondary? Please clarify if this activity has been confirmed by the notifier, including a citation if available.

b. -Please clarify if the specified activity of the enzyme (913,000 units/g) refers to the sum of both monoacyl and diacylglycerol lipase activity or if it is limited to monoacyl glycerol lipase activity.

Response:

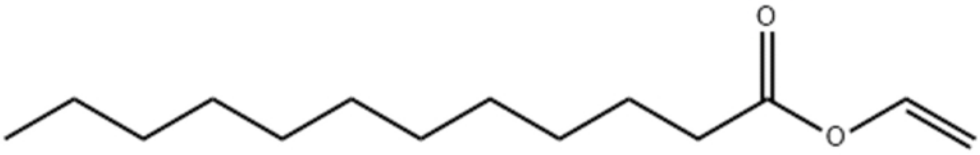
The lipase from *P. camemberti* hydrolyzes mono- and diglycerides with the monoacylglycerol activity being the primary reaction and diacylglycerol activity being secondary.[1] It is typical for there to be secondary reactions that are not reflected in the EC number for enzymes such as acylglycerol lipase (EC No. 3.1.1.23). Note there is no EC number for an enzyme with diglycerol activity as the primary reaction in the list of carboxylic acid ester hydrolase subclass of hydrolases that act on ester bonds.[2]

The activity of Amano's enzyme (913,000 units/g) is assessed by Amano for internal quality control purposes by measuring the basic hydrolysis reaction to the ester bond in vinyl laurate as described in detail in the attached **CONFIDENTIAL** method. The structure of vinyl laurate is as follows:

---

[1] Yamaguchi S, Mase T (1991) Purification and characterization of mono- and diglycerol lipase isolated from *Penicillium camembertii* U-150; Appl Microbiol Biotechnol; 34:720-725. (Copy attached).

[2] https://www.qmul.ac.uk/sbcs/iubmb/enzyme/EC3/1/1/.



Question 3. 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). FDA notes that the assumption of 3 kg food corresponds to the maximum physiological level of consumption for a 60 kg person used in the budget method of estimating exposure (Principles and methods for the risk assessment of chemicals in food. Environmental Health Criteria 240, Chapter 6, updated 2020; https://www.who.int/publications/i/item/9789241572408). Please confirm that your approach is consistent with the budget method approach to confirm our understanding of your assumptions used in the exposure estimate.

Response:

The assumption of 3 kg food corresponds to the maximum physiological level of consumption for a 60 kg person used in the budget method of estimating theoretical overall maximum daily exposure (Hansen, 1966;[3] Hansen, 1979;[4] Douglass et al., 1997)[5], which, as FDA has noted, is discussed in Chapter 6 (Dietary exposure assessment for chemicals in food; updated November 2020) of *Principles and methods for the risk assessment of chemicals in food* (Environmental health criteria; 240), a joint publication of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO).[6] The budget method, however, would yield a lower estimated daily intake (EDI) than we calculated by applying "FDA's standard assumption that an average daily diet consists of 3 kilograms (kg) of

[3] Hansen SC (1966). Acceptable daily intake of food additives and ceiling on levels of use. Food Cosmet Toxicol,4,427–432.

[4] Hansen SC (1979). Conditions for use of food additives based on a budget for an acceptable daily intake. Food Protect, 42,429–432.

[5] Douglass JS, Barraj LM, Tennant DR, Long WR and Chaisson CF (1997). Evaluation of the Budget Method for screening food additive intakes. Food Additives and Contaminants, 14, 791-802.

[6] *See* 6-107 to 6-108.

food per day (including 1.5 kg of beverages) and body weight of 60 kg."[7] Specifically, in addition to not being used in beverages, the enzyme preparation is used only in processed foods, which may be assumed to comprise only 50% of the total intake of solid food. Thus, the EDI calculation in GRN 908 represents a very conservative estimate of daily intake.

* * *

We hope and trust that the information above responds fully to FDA's additional questions regarding Amano's GRN 908. We look forward to the Agency's continued review of the Notice and we would be happy to provide you with any further information you may need.

Sincerely,

Melvin S. Drozen

[7] *See e.g.*, FDA's Guidance for Industry: Preparation of Premarket Submissions for Food Contact Substances (Chemistry Recommendations), December 2007.

6 pages have been removed in accordance with copyright laws. The removed reference is:

Yamaguchi, S., and Mase, T. 1991. "Purification and characterization of mono- and diacylglycerol lipase isolated from *Penicillium camembertii* U-150." *Applied Microbilogy and Biotechnology.*

Viebrock, Lauren

From: Drozen, Melvin S. <Drozen@khlaw.com>
Sent: Thursday, January 28, 2021 10:22 AM
To: Viebrock, Lauren
Cc: Alsobrook, Lisa P.
Subject: [WARNING : MESSAGE ENCRYPTED] GRN 908--Response to FDA Questions
Attachments: Assay method for Lipase activity.pdf

Dear Lauren,

Thank you for the kind wishes and we hope you are well and also off to a good start this year.

This message responds to the request in your January 25, 2021 email below. Specifically, we provided an internal method used to assess lipase activity (designated as confidential) as part of the response to FDA's December 8, 2020 questions regarding GRN 908 for the intended uses of a lipase enzyme preparation derived from *Penicillium camemberti*, and you subsequently asked that we provide a brief narrative to explain how experts could get to a conclusion of safety without the confidential information.

Our client, Amano Enzymes Inc., out of concern that there be no further delay in FDA's review of GRN 908, prefers to lift confidentiality, as opposed to pursuing an effort to maintain confidentiality. Please find the attached assay method for lipase activity without the "confidential" stamp.

We hope and trust that lifting confidentiality for the lipase assay method resolves FDA's additional questions regarding Amano's GRN 908. We look forward to the Agency's continued review of the Notice and will be happy to provide you with any further information you may need.

Best regards,

Mel.

From: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Sent: Monday, January 25, 2021 10:09 PM
To: Drozen, Melvin S. <Drozen@khlaw.com>
Cc: Alsobrook, Lisa P. <alsobrook@khlaw.com>
Subject: RE: GRN 908--Response to FDA Questions

Dear Mel,

Happy new year. Hope the year is starting off well.

Thank you for your email and the responses to our questions regarding GRN 908.

The assay method for lipase activity was designated confidential. Please provide a brief narrative to explain how experts could get to a conclusion of safety without the confidential information.

I am happy to discuss this by phone if you have any questions.

Best,
Lauren

From: Drozen, Melvin S. <Drozen@khlaw.com>
Sent: Tuesday, January 05, 2021 10:47 AM
To: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Cc: Alsobrook, Lisa P. <alsobrook@khlaw.com>
Subject: [WARNING : MESSAGE ENCRYPTED] GRN 908--Response to FDA Questions

Dear Lauren,

Happy New Year. We hope you had a pleasant and safe holiday season.

Please find the attached "Response to FDA's 12-8-2020 request (letter)," which we send on behalf of our client, Amano Enzyme Inc. (Amano), to address the questions posed by the Food and Drug Administration (FDA), attached to your December 8, 2020 email below, regarding the Generally Recognized as Safe (GRAS) notice that we submitted on January 14, 2020 to cover the intended uses of a lipase enzyme preparation derived from *Penicillium camemberti*. References discussed in our response letter are also attached: (1) Yamaguchi (1991), and (2) Assay method for lipase activity (Confidential internal method).

We look forward to the FDA's continued review of GRN 908.

Sincerely,

Mel and Lisa.

Melvin S. Drozen
Partner
tel: +1 202.434.4222 | fax: +1 202.434.4646 | drozen@khlaw.com
1001 G Street NW, Suite 500 West | Washington, DC 20001

From: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Sent: Tuesday, December 8, 2020 1:14 PM
To: Drozen, Melvin S. <Drozen@khlaw.com>
Cc: Alsobrook, Lisa P. <alsobrook@khlaw.com>
Subject: RE: GRN 908--Response to FDA Questions

Dear Mr. Drozen,

Thank you for the information you submitted in response to our questions for GRN 000908. We have an additional questions to be addressed that are attached to this email.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Regards,
Lauren

Lauren VieBrock
Regulatory Review Scientist/Microbiology Reviewer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 301-796-7454
lauren.viebrock@fda.hhs.gov

From: Drozen, Melvin S. <Drozen@khlaw.com>
Sent: Thursday, December 03, 2020 10:09 AM
To: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Cc: Alsobrook, Lisa P. <alsobrook@khlaw.com>
Subject: GRN 908--Response to FDA Questions

Dear Lauren,

Please find the attached "GRN 908 (response letter)," which we send on behalf of our client, Amano Enzyme, Inc. (Amano), to address the questions posed by the Food and Drug Administration (FDA), attached to your November 12, 2020 email below, regarding the Generally Recognized as Safe (GRAS) notice that we submitted on January 14, 2020 to cover the intended uses of a lipase enzyme preparation derived from *Penicillium camemberti*.

We look forward to the FDA's continued review of GRN 908.

Sincerely,

Mel Drozen and Lisa Alsobrook.

From: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Sent: Thursday, November 12, 2020 1:20 PM
To: Drozen, Melvin S. <Drozen@khlaw.com>
Subject: GRN 908 Questions

Dear Mr. Drozen,

During our review of GRAS Notice No. 000908, we noted questions that need to be addressed and are attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Regards,
Lauren

Lauren VieBrock
Regulatory Review Scientist/Microbiology Reviewer
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 301-796-7454
lauren.viebrock@fda.hhs.gov

Viebrock, Lauren

From: Drozen, Melvin S. <Drozen@khlaw.com>
Sent: Monday, February 8, 2021 12:35 PM
To: Viebrock, Lauren
Cc: Alsobrook, Lisa P.; Fulmer, Preston A.
Subject: RE: GRN 000908
Attachments: 2021_02_05 No Questions Letter draft GRN 908 Transmittal.pdf

Dear Lauren,

Thanks very much. We will review it of course and let you know if we have any questions.

Regards,

Mel.

Melvin S. Drozen
Partner
tel: +1 202.434.4222 | fax: +1 202.434.4646 | drozen@khlaw.com
1001 G Street NW, Suite 500 West | Washington, DC 20001

From: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Sent: Monday, February 8, 2021 11:52 AM
To: Drozen, Melvin S. <Drozen@khlaw.com>
Subject: GRN 000908

Dear Mr. Drozen,

Please find attached our response letter for GRAS Notice No. GRN 000908. Please let me know if you have any questions.

Regards,
Lauren VieBrock

Assay method for Lipase activity (LV method)

Solutions

1) 0.05 mol/L Hydrochloric acid solution

Dilute 1 L of 0.1 mol/L Hydrochloric acid (reagent) to 2 L with water.

2) Ethanol -Acetone mixture(1:1)

Add 5 L of Ethanol(95) to 5 L of Acetone, and mix.

3) 0.05 mol/L Sodium hydroxide solution

Dilute 1 L of 0.1 mol/L Sodium hydroxide solution (reagent) to 2 L with boiled water.

4) PVA solution

Dissolve 40.0 g of Poly vinyl alcohol (saponification degree : 98.8±0.2 mol%) in about 1600 mL of water while heating at 75～80 ℃, and cool. If needed, filtrate with cotton. Dilute to 2000 mL with water.

5) Phenolphthalein solution

Dissolve 1.0 g of Phenolphthalein in 100 mL of Ethanol (95).

6) 0.05 mol/L Citric acid solution

Dissolve 2.10 g of Citric acid monohydrate in 150 mL of water, and dilute to 200 mL with water.

7) 0.1 mol/L Disodium hydrogenphosphate solution

Dissolve 28.39 g of Disodium hydrogenphosphate in 1 L of water, and dilute to 2 L with water.

8) McIlvaine buffer pH 5.6

Add 0.1 mol/L Disodium hydrogenphosphate solution to 0.05 mol/L Citric acid solution until the pH stabilizes at 5.60.

9) Substrate solution

Mix 194 mL of PVA solution and 6mL of Vinyl Laurate, and cool below 10 ℃ with iced water. Homogenize at 14500±300 rpm for 10 minutes in total by repeatedly mixing for 10/3 minutes and leaving at rest for 10/3 minutes cooling. Store it for 1 hour at 2～8 ℃.

Preparation of the sample solution

Dissolve the sample in an appropriate amount of water or a buffer solution. This enzyme solution should be prepared to make the value of (T_0-T_{30}) into 0.7～1.7.

PROCEDURE

Assay

Pipet 4 mL of McIlvaine buffer pH 5.6 and 5 mL of Substrate solution into test tubes, and mix. Place the tubes in water bath maintained at 40±0.5 ℃ for 10 minutes. Add 1 mL of Sample solution and mix, place the tubes in water bath at 40±0.5 ℃ for exactly 30 minutes. Add 10 mL of Ethanol-Acetone mixture(1:1) and mix, place the tubes in running water for 10 minutes. Add 10 mL of 0.05 mol/L Sodium hydroxide solution and 10 mL of Ethanol-Acetone mixture(1:1) and 2 drops of Phenolphthalein solution. Titrate with 0.05 mol/L Hydrochloric acid solution until the pH reaches at 10.00 while stirring. (Reaction solution)

As the blank, pipet 4 mL of McIlvaine buffer pH 5.6 and 5 mL of Substrate solution into test tubes, and mix. Place the tubes in water bath at 40±0.5 ℃ for 30 minutes. Add 10 mL of Ethanol-Acetone mixture(1:1) and mix, place the tubes in running water for 10 minutes. Add 1 mL of Sample solution and mix. Add 10 mL of 0.05 mol/L Sodium hydroxide solution and 10 mL of Ethanol-Acetone mixture(1:1) and 2 drops of Phenolphthalein solution. Titrate with 0.05 mol/L Hydrochloric acid solution until the pH reaches at 10.00 while stirring. (Blank solution)

Definition of Activity Unit

One unit is defined as the quantity of enzyme required to liberate 1 μmol of fatty acid per 1 minute under the conditions of the assay.

CALCULATION

$$\text{Lipase activity unit/g} = (T_0 - T_{30}) \times f \times 50 / 30 \times n$$

T_0 : titration Volume of Blank solution, in mL
T_{30} : titration volume of Reaction solution, in mL
f : factor of 0.05 mol/L Hydrochloric acid solution
50 : quantity of fatty acid equivalent to 1 mL of 0.05 mol/L Hydrochloric acid solution, in μmole
30 : Reaction time (minutes)
n : Dilution factor of the enzyme

Assay method for Lipase activity (LV method)

Solutions

1) 0.05 mol/L Hydrochloric acid solution

Dilute 1 L of 0.1 mol/L Hydrochloric acid (reagent) to 2 L with water.

2) Ethanol -Acetone mixture(1:1)

Add 5 L of Ethanol(95) to 5 L of Acetone, and mix.

3) 0.05 mol/L Sodium hydroxide solution

Dilute 1 L of 0.1 mol/L Sodium hydroxide solution (reagent) to 2 L with boiled water.

4) PVA solution

Dissolve 40.0 g of Poly vinyl alcohol (saponification degree : 98.8±0.2 mol%) in about 1600 mL of water while heating at 75～80 ℃, and cool. If needed, filtrate with cotton. Dilute to 2000 mL with water.

5) Phenolphthalein solution

Dissolve 1.0 g of Phenolphthalein in 100 mL of Ethanol(95).

6) 0.05 mol/L Citric acid solution

Dissolve 2.10 g of Citric acid monohydrate in 150 mL of water, and dilute to 200 mL with water.

7) 0.1 mol/L Disodium hydrogenphosphate solution

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Preparation of the sample solution

Dissolve the sample in an appropriate amount of water or a buffer solution. This enzyme solution should be prepared to make the value of (T_0-T_{30}) into 0.7～1.7.

PROCEDURE

Assay

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50 : quantity of fatty acid equivalent to 1 mL of 0.05 mol/L Hydrochloric acid solution, in μmole
30 : Reaction time (minutes)
n : Dilution factor of the enzyme