Celia Martin, Ph.D.
Lallemand Inc.
1620 Prefontaine Street
H1W 2N8, Montreal, QC
CANADA

Re: GRAS Notice No. GRN 001047

Dear Dr. Martin:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001047. We received Lallemand Inc. (Lallemand)'s notice on September 22, 2021, and filed it on March 11, 2022. Lallemand submitted an amendment to the notice on April 4, 2023, containing additional information on enzyme identity, manufacturing, specifications, and analytical methods.

The subject of the notice is lipase enzyme preparation produced by *Saccharomyces cerevisiae* expressing a gene encoding a lipase from *Fusarium oxysporum* (lipase enzyme preparation) for use as an enzyme at up to 29.8 mg Total Organic Solids (TOS)/kg flour in the manufacture of baked goods. The notice informs us of Lallemand's view that this use of lipase enzyme preparation is GRAS through scientific procedures.

Commercial enzyme preparations that are used in food processing typically contain an enzyme component that catalyzes the chemical reaction as well as substances used as stabilizers, preservatives, or diluents. Enzyme preparations may also contain components derived from the production organism and from the manufacturing process, e.g., constituents of the fermentation media or the residues of processing aids. Lallemand's notice provides information about the components in the lipase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, lipase is identified by the Enzyme Commission Number 3.1.1.3,[1] and the Chemical Abstracts Service Number 9001-62-1. Lallemand states that the primary amino acid sequence of the lipase consists of 331 amino acids and has a calculated molecular weight of 35 kDa.

Lallemand states that the *S. cerevisiae* production organism is a non-pathogenic and non-toxigenic yeast with a history of safe use in food production.

[1] https://iubmb.qmul.ac.uk/enzyme/EC3/1/1/3.html

Lallemand states that the *S. cerevisiae* production strain DSM 34129, referred to as LALL-LI by Lallemand, was constructed from the host strain by targeted integration of an expression cassette carrying a lipase gene from *F. oxysporum* under control of a promoter and a terminator from *S. cerevisiae* and an expression cassette carrying the genes for two *S. cerevisiae* chaperone proteins from the parent strain for overexpression.[2] Lallemand states that whole genome sequencing was used to confirm the sequence integrity of the inserted sequences and PCR was used to confirm genetic stability. Lallemand verified that the final production strain does not contain any functional or transferable antibiotic resistance genes by genome sequencing.

Lallemand states that the lipase enzyme preparation is manufactured by controlled fermentation of a pure culture of the *S. cerevisiae* production strain. The enzyme is secreted into the fermentation medium. After fermentation, the medium containing the enzyme is separated from the biomass, recovered, and concentrated by a series of filtration and ultrafiltration steps. The resulting lipase enzyme concentrate is formulated to a liquid preparation with stabilization and preservation agents including sucrose, glycerol, sodium chloride, potassium sorbate and sodium benzoate, or a solid preparation by the addition of carriers, such as salt, starch, or dextrin, and dried. Lallemand states that the entire process is performed in accordance with current Good Manufacturing Practices and with raw materials that are food-grade. Lallemand also states that lipase enzyme preparation does not contain any major food allergens from the fermentation media.

Lallemand has established food-grade specifications and states that the lipase enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 13th edition, 2022), and to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing established by the FAO/WHO Joint Expert Committee on Food Additives (JECFA, 2006). Lallemand provides data from analyses of three batches of lipase enzyme preparation to demonstrate that the manufacturing acceptance criteria have been met, including the absence of the production organism in the final product.

Lallemand intends to use lipase enzyme preparation at a maximum level of 29.8 mg TOS/kg flour to catalyze the hydrolysis of triglyceride ester bonds into diglycerides and subsequently into monoglycerides and glycerol, as well as free fatty acids. Lallemand notes that the lipase enzyme is inactivated during food production. Lallemand estimates a maximum dietary exposure to the lipase enzyme preparation to be 0.373 TOS/kg bw/day from the use in baked goods with the assumption that the added lipase enzyme preparation remains present in the final food.[3]

Lallemand relies on published information that discusses the safety of the *S. cerevisiae*

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[2] Lallemand states that the production strain is deposited at the Deutsche Sammlung von Mikroorganismen and Zellkulturen as DSM 34129.

[3] Lallemand uses the Budget method to estimate the dietary exposure to lipase enzyme preparation based on the consumption of 50 g of solid foods per kg bw/d. Lallemand assumes that 25% of the solid foods (12.5 g/kg bw/d) will be baked goods and contain the lipase enzyme preparation at the recommended use level.

production organism, the safety of microbial enzyme preparations used in food processing, the safety of the *F. oxysporum* donor organism, and the safety of lipase enzymes produced by different species of microorganisms, including lipase derived from *F. oxysporum*.

Lallemand discusses publicly available literature, as well as the conclusions of several organizations and working groups, about the low risk of allergenicity posed by enzymes from their intended use, to address potential allergenicity due to lipase. Based on bioinformatic analyses using criteria recommended by FAO/WHO (FAO/WHO, 2001; Codex Alimentarius, 2009; JECFA, 2016), Lallemand reports no exact matches using a window of 80 amino acids or a window of 8 amino acids. Based on the totality of the information available Lallemand concludes that it is unlikely that oral consumption of lipase will result in allergenic responses from its intended uses.

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

Standards of Identity

In the notice, Lallemand states its intention to use lipase enzyme preparation in several food categories, including foods for which standards of identity exist, located in Title 21 of the Code of Federal Regulations. We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity.

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

Section 301(ll) of the FD&C Act prohibits the introduction or delivery for introduction into interstate commerce of any food that contains a drug approved under section 505 of the FD&C Act, a biological product licensed under section 351 of the Public Health Service Act, or a drug or a biological product for which substantial clinical investigations have been instituted and their existence made public, unless one of the exemptions in section 301(ll)(1)-(4) applies. In our evaluation of Lallemand's notice concluding that lipase enzyme preparation is GRAS under its intended conditions of use, we did not consider whether section 301(ll) or any of its exemptions apply to foods containing lipase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing lipase enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Lallemand provided, as well as other information available to FDA, we have no questions at this time regarding Lallemand's conclusion that lipase enzyme preparation produced by *S. cerevisiae* expressing a gene encoding a lipase from *F. oxysporum* is GRAS under its intended conditions of use. This letter is not an affirmation that lipase enzyme preparation produced by *S. cerevisiae* expressing a gene encoding a lipase from *F. oxysporum* is GRAS under 21 CFR 170.35. Unless

noted above, our review did not address other provisions of the FD&C Act. Food ingredient manufacturers and food producers are responsible for ensuring that marketed products are safe and compliant with all applicable legal and regulatory requirements.

In accordance with 21 CFR 170.275(b)(2), the text of this letter responding to GRN 001047 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2023.05.12 16:49:30 -04'00'

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