U.S. FOOD & DRUG ADMINISTRATION

Human Foods Program

Joab Trujillo
AB Enzymes Inc.
8211 W. Broward Blvd. Suite 420
Plantation, FL 33324

Re: GRAS Notice No. GRN 001196

Dear Mr. Trujillo:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001196. We received AB Enzymes Inc. (AB Enzymes)'s notice on July 1, 2024 and filed it on September 10, 2024. AB Enzymes submitted an amendment to the notice on January 16, 2025, containing additional information on enzyme identity, the enzyme safety narrative, and specifications.

The subject of the notice is triacylglycerol lipase enzyme preparation produced by *Trichoderma reesei* expressing a gene encoding an engineered triacylglycerol lipase from *Thermomyces lanuginosus* (lipase enzyme preparation) for use as an enzyme at up to 4 mg Total Organic Solids (TOS)/kg flour in the production of baked goods and other cereal based products. The notice informs us of AB Enzymes' 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. AB Enzymes' 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. AB Enzymes states that the primary amino acid sequence of the lipase consists of 274 amino acids with a molecular weight of 30 kDa.

AB Enzymes states that the *T. reesei* production organism is a non-pathogenic and non-toxigenic fungus with a history of safe use in food production.

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

AB Enzymes states that the *T. reesei* production strain "AR-822" was constructed from the host strain by targeted integration of an expression cassette carrying a gene encoding an engineered lipase[2] from *T. lanuginosus* under control of a promoter and a terminator from *T. reesei* and a selectable marker. AB Enzymes states that whole genome sequencing was used to confirm the sequence integrity of the production strain. AB Enzymes states that the final production strain does not contain any functional or transferable antibiotic resistance genes.

AB Enzymes states that lipase enzyme preparation is manufactured by controlled fermentation of a pure culture of the *T. reesei* 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 an enzyme preparation with sunflower oil and maltodextrin. The final lipase enzyme preparation is a light beige to light brown powder. AB Enzymes states that the entire process is performed in accordance with current Good Manufacturing Practices and with food-grade raw materials.

AB Enzymes has established food-grade specifications including a limit for lead (< 0.5 mg/kg) 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). AB Enzymes provides results from analyses of three non-consecutive batches of lipase enzyme concentrate to demonstrate that the manufacturing acceptance criteria can be met, including the absence of the production organism in the final product.

AB Enzymes intends to use lipase enzyme preparation at a maximum level of 4 mg TOS/kg flour in the production of baked goods and other cereal based products to catalyze the hydrolysis of ester bonds of triacylglycerols (at the position 1 and 3 of the glycerol molecule), resulting in the formation of mono- and diacylglycerols, free fatty acids and, in some cases, also glycerol. AB Enzymes notes that the lipase enzyme is inactivated during food production. AB Enzymes estimates a maximum dietary exposure to the lipase enzyme preparation to be 0.036 mg TOS/kg bw/day from the use in food and drinks with the assumption that the added lipase enzyme preparation remains present in the final food.[3]

AB Enzymes relies on published information that discusses the safety of the *T. reesei* production organism, the safety of microbial enzyme preparations used in food

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[2] The gene encodes a *T. lanuginosus* lipase enzyme that includes 15 amino acid substitutions compared to wild-type lipase, which is the subject of GRN 000043, and one additional amino acid substitution compared to the lipase that is the subject of GRN 000103, which has 14 additional amino acid substitutions compared to wild-type.

[3] AB Enzymes uses the Budget method to estimate the dietary exposure to lipase enzyme preparation based on the consumption of 12.5 g of solid foods per kg bw/d (worst case scenario) containing the lipase enzyme preparation at the recommended use level.

processing, the safety of the *T. lanuginosus* donor organism, and the safety of lipase enzymes. In addition, AB Enzymes states that enzymes are generally added at the lowest level to catalyze the desired reaction and that exposure is generally low. In support of the safety of the lipase enzyme preparation, AB Enzymes summarizes the available published literature that supports the history of safe use of lipase in food. AB Enzymes also discusses the amino acid sequence and protein structural analysis comparisons between the lipase from *T. lanuginosus* and the lipases discussed in GRN 000043 and GRN 000103.[4] AB Enzymes states that given the high degree of structural similarity between the lipases, they conclude that the notified lipase enzyme preparation has a similar safety profile to the lipases that were the subjects of GRN 000043 and GRN 000103. AB Enzymes discusses the results of toxicological studies using the lipase enzyme from GRN 000103, which show no treatment related effects, to support safety of the notified lipase. Also, AB Enzymes states that a literature search did not identify any information that would contradict a general recognition of safety of the lipase enzyme preparation.

AB Enzymes 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), AB Enzymes reports that no sequence homology of *T. lanuginosus* lipase to known allergens that would raise allergenicity concerns were identified. Based on the totality of the information available, AB Enzymes 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, AB Enzymes concludes that lipase enzyme preparation is GRAS for its intended use.

Standards of Identity

In the notice, AB Enzymes 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 CFR. 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.

[4] GRN 000043 and GRN 000103 describe the intended food uses of lipase enzyme preparation. We evaluated GRN 000043 and responded with a letter on September 22, 2000, stating that we had no questions at that time regarding Novo Nordisk BioChem North America, Inc.'s GRAS conclusion. We evaluated GRN 000103 and responded with a letter on August 19, 2002, stating that we had no questions at that time regarding Novozymes North America, Inc.'s GRAS conclusion.

Allergen Labeling

The Federal Food, Drug, and Cosmetic Act (FD&C Act) requires that the label of a food that is or contains an ingredient that contains a "major food allergen" declare the allergen's presence (section 403(w)). The FD&C Act defines a "major food allergen" as one of nine foods or food groups (i.e., milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, soybeans, and sesame) or a food ingredient that contains protein derived from one of those foods. Lipase enzyme preparation may require labeling under the FD&C Act because it may contain protein derived from wheat. Questions about petitions or notifications for exemptions from the food allergen labeling requirements should be directed to the Division of Food Ingredients in the Office of Pre-Market Additive Safety. Questions related to food labeling in general should be directed to the Office of Nutrition and Food Labeling in the Nutrition Center of Excellence.

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 AB Enzymes' 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 AB Enzymes provided, as well as other information available to FDA, we have no questions at this time regarding AB Enzymes' conclusion that lipase enzyme preparation is GRAS under its intended conditions of use. This letter is not an affirmation that lipase enzyme preparation is GRAS under 21 CFR 170.35. Unless noted above, our review did not address other provisions of the FD&C Act. Food ingredient manufacturers and food producers are responsible for ensuring that marketed products are safe and compliant with all applicable legal and regulatory requirements.

In accordance with 21 CFR 170.275(b)(2), the text of this letter responding to GRN 001196 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2025.03.03 16:54:10 -05'00'

Susan Carlson, Ph.D.
Director
Division of Food Ingredients
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