Joab Trujillo
AB Enzymes Inc.
8211 W. Broward Blvd., Suite 375
Plantation, FL 33324

Re: GRAS Notice No. GRN 000981

Dear Mr. Trujillo:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000981. We received AB Enzymes Inc. (AB Enzymes)'s GRAS notice on November 17, 2020 and filed it on May 5, 2021. AB Enzymes submitted amendments to the notice on March 11, 2022, April 14, 2022, and May 6, 2022 that provided additional information on the safety of the production strain, analytical methods used to determine specifications, the safety narrative, and references.

The subject of the notice is sterol esterase enzyme preparation produced by *Trichoderma reesei* expressing a gene encoding sterol esterase from *Melanocarpus albomyces* (sterol esterase enzyme preparation) for use as an enzyme at up to 10 mg Total Organic Solids (TOS)/kg flour used in the manufacture of baked goods. The notice informs us of AB Enzymes' view that this use of sterol esterase 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 sterol esterase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, sterol esterase is identified by the Chemical Abstracts Service number 9026-00-0 and the Enzyme Commission Number 3.1.1.13.[1] AB Enzymes states the mature enzyme of sterol esterase consists of 558 amino acids and has a calculated molecular mass of 59 kDa.

AB Enzymes states that the *T. reesei* production organism is non-pathogenic and non-

[1] https://www.qmul.ac.uk/sbcs/iubmb/enzyme/EC3/1/1/13.html

toxigenic. AB Enzymes states that the recipient strain[2] used in the construction of the production strain, AR 777, was transformed with an expression cassette carrying the gene encoding sterol esterase from *M. albomyces* fused to a carrier protein.[3] AB Enzymes states it confirmed the sequence integrity by Southern blot. AB Enzymes evaluated the genetic stability of the production strain by monitoring production of sterol esterase. AB Enzymes states that the production strain does not contain any functional or transmissible antibiotic resistance genes.

AB Enzymes states that the sterol esterase enzyme preparation is manufactured by submerged fermentation of a pure culture of the *T. reesei* AR-777 production strain under controlled conditions. The sterol esterase enzyme is secreted into the medium, recovered by filtration or centrifugation, and concentrated. The enzyme is then subjected to polish and germ filtration to produce an enzyme concentrate free of any insoluble materials and residual production strain. The enzyme concentrate is formulated with sunflower oil and wheat flour to produce a light beige sterol esterase enzyme preparation. AB Enzymes states that the entire process is performed using food grade raw materials and in accordance with current good manufacturing practices.

AB Enzymes has established food grade specifications and states that the sterol esterase 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 data from analyses of three batches of sterol esterase enzyme concentrate to demonstrate that the manufacturing acceptance criteria have been met, including the absence of the production organism and antibiotic activity.

AB Enzymes intends to use sterol esterase enzyme preparation at a maximum use level of 10 mg TOS/kg flour to hydrolyze plant lipids during the manufacture of baked goods. AB Enzymes notes that the sterol esterase enzyme preparation will be added to flour, prior to the dough mixing stage, and inactivated during baking. AB Enzymes, however, estimates a maximum dietary exposure to sterol esterase enzyme preparation to be 0.0888 mg TOS/kg body weight per day (mg TOS/kg bw/d) from intended uses with the assumption that all the sterol esterase will remain in the final food.[4]

AB Enzymes relies on published information to demonstrate the safety of the *T. reesei*

---

[2] AB Enzymes states that the recipient *T. reesei* strain RF10310 was derived from the parental *T. reesei* strain QM6a, which was characterized by the Centraalbureau voor Schimmelcultures (CBS) in the Netherlands.

[3] AB Enzymes discusses transformation with a plasmid carrying an expression cassette containing the *M. albomyces* sterol esterase gene, the carbohydrate binding domain of the *T. reesei* cellobiohydrolase 2 gene for use as a carrier protein, *T. reesei* promoter and terminator, and a synthetic acetamidase gene selectable marker from *Aspergillus nidulans*. AB enzymes state that the carrier protein is the native cellobiohydrolase 2. It is used to improve the production of sterol esterase and does not have any enzymatic activity in the final sterol enzyme preparation.

[4] AB Enzymes uses the Budget method to estimate dietary exposure to sterol esterase enzyme preparation based on consumption of a maximum of 12.5 g of processed solid foods/kg bw/d and a ratio of raw material to final food (baked good) of 0.71.

production organism and the safety of microbial enzyme preparations used in food processing. Based on results from bacterial reverse mutation, *in vitro* mammalian chromosomal aberration, and 13-week oral toxicity study in rats performed on a related *T. reesei* production strain, AB Enzymes states that their production strain lineage lacks potential for toxicity. AB Enzymes did not detect any mycotoxins, toxic secondary metabolites, or antibiotic activity based on results of batch analyses of the sterol esterase enzyme concentrate.

AB Enzymes discusses publicly available literature, as well as the conclusions of several organizations and working groups concerning the low risk of allergenicity posed by oral consumption of enzymes, to address the potential for allergenicity to sterol esterase. Based on bioinformatic analyses, AB Enzymes reports no matches between the amino acid sequences of the sterol esterase and those of known allergens based on the guidelines developed by Codex Alimentarius Commission (FAO, 2009). Based on the totality of the information available, AB Enzymes concludes that it is unlikely that oral consumption of sterol esterase enzyme from the intended use will result in allergenic responses. In addition, AB Enzymes addresses the safety of their sterol esterase by discussing its similar activity to known food enzymes, safety of the manufacturing steps for commercial enzyme preparations, and the expected gastrointestinal fate of enzymes added to food.

Based on the data and information summarized above, AB Enzymes concludes that sterol esterase enzyme preparation is GRAS for its intended use.

Standards of Identity

In the notice, AB Enzymes states its intention to use sterol esterase 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.

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 (effective January 1, 2023)) or a food ingredient that contains protein derived from one of those foods. Sterol esterase enzyme preparation requires labeling under the FD&C Act because it contains protein derived from wheat.

Section 301(ll) of the 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 sterol esterase 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 sterol esterase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing sterol esterase 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 sterol esterase enzyme preparation produced by *T. reesei* expressing a gene encoding sterol esterase from *M. albomyces* is GRAS under its intended conditions of use. This letter is not an affirmation that sterol esterase enzyme preparation produced by *T. reesei* expressing a gene encoding sterol esterase from *M. albomyces* 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 000981 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2022.05.10 14:51:56 -04'00'

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