U.S. FOOD & DRUG ADMINISTRATION

Human Foods Program

Joab Trujillo
8211 W. Broward Blvd Suite 420
Plantation, Florida 33324

Re: GRAS Notice No. GRN 001197

Dear Mr. Trujillo

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001197. We received AB Enzymes, Inc.'s (AB Enzymes) notice on July 15, 2024 and filed it on September 17, 2024. AB Enzymes submitted amendments to the notice on December 17, 2024, and February 24, 2025, that provided clarifications on identity, specifications, manufacturing, and the safety narrative.

The subject of the notice is fructanase enzyme preparation produced by *Trichoderma reesei* expressing fructanase from *Lactobacillus crispatus* (fructanase enzyme preparation) for use as an enzyme at up to 12 mg total organic solids (TOS)/kg raw material in baking and cereal based products. The notice informs us of AB Enzymes' view that this use of fructanase 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 fructanase enzyme preparation.

Fructanase is identified by the Chemical Abstracts Service number 37288-56-5 and the Enzyme Commission Number 3.2.1.80.[1] AB Enzymes states that the primary sequence of fructanase consists of 1081 amino acids with a calculated molecular weight of 121 kDa for the mature enzyme protein and 88 kDa for the truncated enzyme protein.

AB Enzymes states that the *T. reesei* production organism is non-pathogenic and non- toxigenic and is a well-characterized production organism with history of safe use in the food industry. AB Enzymes states that the production strain, AR-577, was constructed through transformation with an expression cassette carrying a modified, synthetic

[1] https://iubmb.qmul.ac.uk/enzyme/EC3/2/1/80.html

fructanase gene from *Lactobacillus crispatus*.[2] AB Enzymes states that they confirmed sequence integration by Southern blot analysis and whole genome sequencing. AB Enzymes confirmed the genetic stability of the production strain by monitoring enzyme activity and Southern blot analysis. AB Enzymes verified the absence of functional or transferable antibiotic resistance genes in the final production strain genome.

AB Enzymes states that the fructanase enzyme preparation is produced by submerged fed-batch fermentation of a pure culture of the *T. reesei* production strain under controlled conditions. The fructanase enzyme is secreted into the fermentation medium and then recovered by an initial pre-treatment and physical separation step, concentration via filtration, and a polish filtration. The enzyme preparation is then standardized to the desired activity level and formulated with other ingredients, including sunflower oil and wheat flour, resulting in a light beige powder. 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 including lead at < 0.5 mg/kg and states that the fructanase enzyme preparation conforms to the specifications set 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 fructanase enzyme concentrate to demonstrate that the manufacturing acceptance criteria can be met, including the absence of the production organism.

AB Enzymes intends to use fructanase enzyme preparation at a maximum use level of 12 mg total organic solids (TOS)/ kg raw material as the powder form in baking and cereal based products. AB Enzymes states that fructanase catalyzes the hydrolysis of fructooligosaccharides and related polysaccharides. AB Enzymes notes that the final enzyme is denatured during the baking process. AB Enzymes estimates a maximum dietary exposure to fructanase enzyme preparation to be 0.107 mg TOS/kg body weight per day (mg TOS/kg bw/d) from the intended uses and assumes that all of the fructanase enzyme preparation will be active and remain in the final food.[3]

In support of the safety of the fructanase enzyme preparation, AB Enzymes highlights published information that supports the history of safe use of fructanase in food. AB Enzymes states that enzymes are generally added at the lowest level to catalyze the desired reaction, and that exposure is generally low. AB Enzymes notes that enzymatic side activities, common in food enzyme preparations, are present at very low levels and do not pose a safety concern. AB Enzymes states that the fructanase derived from *L. crispatus* contains two modifications but notes that neither modification is expected to alter the safety profile of the enzyme. In support of the safety of the fructanase enzyme

[2] AB Enzymes states that the modified *L. crispatus* includes a changed amino acid and a truncated amino acid sequence; AB Enzymes notes that the expression cassette also included an *amdS* marker gene for selection of transformants.
[3] AB enzymes uses the Budget method to estimate the dietary exposure to fructanase enzyme preparation based on consumption of 12.5 g of solid foods per kg bw/d (worst case scenario) containing the fructanase enzyme preparation at the maximum recommended use level.

preparation, AB Enzymes summarizes corroborative, unpublished toxicological studies on the notified fructanase enzyme preparation. This includes a bacterial reverse mutation assay, an *in vitro* micronucleus assay, and a 90-day repeated dose oral toxicity study in rats. AB Enzymes states that the fructanase enzyme preparation was not mutagenic, and there were no treatment-related adverse effects up to the highest dose tested (1000 mg TOS/kg bw/d). AB Enzymes calculates a margin of exposure to be 9,346 using the No Observed Adverse Effect Level (NOAEL) and the estimated dietary exposure for the intended uses of the fructanase enzyme preparation.[4] AB Enzymes relies on published information that discusses the safety of the *T. reesei* production organism. Additionally, AB Enzymes states that a literature search, conducted through May 2024, did not identify any information that would contradict a general recognition of safety of the fructanase enzyme preparation.

AB Enzymes discusses publicly available literature to address potential allergenicity due to fructanase. Based on bioinformatic analysis, AB Enzymes reports no significant matches between the amino acid sequences of the fructanase and the primary sequences of known food allergens based on the guidelines developed by the Codex Alimentarius Commission (Codex, 2009). Based on the totality of information available, AB Enzymes concludes that it is unlikely that oral consumption of fructanase enzyme preparation from the intended uses will result in allergic responses.

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

Standards of Identity

In the notice, AB Enzymes states its intention to use fructanase enzyme preparation in a food category 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 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. The fructanase preparation requires labeling under the FD&C Act because it is formulated with wheat flour.

[4] FDA notes that the margin of safety stated by the notifier is based on unpublished safety studies and is corroborative of the published information regarding enzyme preparations used in food processing.

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 fructanase 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 fructanase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing fructanase 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 fructanase enzyme preparation is GRAS under its intended conditions of use. This letter is not an affirmation that fructanase 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 001197 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

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Susan J. Carlson, Ph.D. Director
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