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

Re: GRAS Notice No. GRN 001173

Dear Mr. Trujillo:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001173. We received AB Enzymes Inc. (AB Enzymes)'s notice on February 24, 2024, and filed it on March 7, 2024. AB Enzymes submitted amendments to the notice on May 1, 2024 and July 9, 2024 containing additional information on enzyme identity, the enzyme safety narrative, and specifications.

The subject of the notice is invertase enzyme preparation produced by *Trichoderma reesei* expressing a gene for invertase from *Aspergillus niger* (invertase enzyme preparation) for use as an enzyme at up to 7 mg Total Organic Solids (TOS)/kg sucrose in the production of short chain fructooligosaccharides (sc-FOS) and up to 14 mg TOS/kg raw material in fruit and vegetable processing. The notice informs us of AB Enzymes' view that this use of invertase 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 invertase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, invertase is identified by the Enzyme Commission Number 3.2.1.26,[1] and the Chemical Abstracts Service Number 9001-57-4. AB Enzymes states that the primary amino acid sequence of the invertase consists of 628 amino acids with a calculated molecular weight of 66 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/EC3/2/1/26.html

AB Enzymes states that the *T. reesei* production strain "AR-996" was constructed from the host strain by targeted integration of an expression cassette carrying an invertase gene from *A. niger* 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 invertase enzyme preparation is manufactured by controlled fed-batch submerged fermentation of a pure culture of the *T. reesei* AR-966 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 invertase enzyme concentrate is brown and is formulated to a liquid enzyme preparation with glycerol, trisodium citrate, citric acid, and water. AB Enzymes states that the entire process is performed in accordance with current Good Manufacturing Practices and with food-grade raw materials. AB Enzymes also states that the invertase enzyme preparation does not contain any major food allergens.

AB Enzymes has established food-grade specifications including a limit for lead (< 0.05 mg/kg) and states that the invertase 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 invertase enzyme concentrate to demonstrate that the manufacturing acceptance criteria have been met, including the absence of the production organism and antibiotic activity in the final product.

AB Enzymes intends to use invertase enzyme preparation at a maximum level of 7 mg TOS/kg sucrose and at a maximum level of 14 mg TOS/kg raw material in fruit and vegetable processing to catalyze the breakdown of sucrose to fructose and glucose, utilizing the hydrolysis of terminal non-reducing β-D-fructofuranoside residues in β-D-fructofuranosides for the production of sc-FOS and sugar reduction in fruit and vegetable processing (i.e., purees and juices). AB Enzymes notes that the invertase enzyme is inactivated during food production. AB Enzymes estimates a maximum dietary exposure to the invertase enzyme preparation to be 0.63 mg TOS/kg bw/day from the use in food and drinks with the assumption that the added invertase enzyme preparation remains present in the final food.[2]

AB Enzymes relies on published information that discusses the safety of the invertase enzyme, the *T. reesei* production organism, the *A. niger* donor organism, and the safety of microbial enzyme preparations used in food processing. In addition, AB Enzymes states that enzymes are generally added at the lowest level to catalyze the desired

[2] AB Enzymes uses the Budget method to estimate the dietary exposure to invertase enzyme preparation based on the consumption of 12.5 g of solid foods and 25 mL of beverages per kg bw/d (worst case scenario) containing the invertase enzyme preparation at the recommended use level.

reaction and that exposure is generally low. In support of the safety of the invertase enzyme preparation, AB Enzymes summarizes the available published literature that supports the history of safe use of invertase in food, and references prior data and information on invertases discussed in GRN 000088, GRN 000537 and GRN 001006.[3] In the amendment dated May 1, 2024, AB Enzymes provides additional information on the similarities and differences between the invertase enzyme preparation and these other invertases sourced from related and non-related microorganisms. This includes sequence and protein structural analysis comparisons between the invertase from *A. niger* and invertases sourced from *Aspergillus fijiensis* and *Saccharomyces cerevisiae*. AB Enzymes states that given the high degree of structural similarity between the invertases, they conclude that the notified invertase enzyme preparation would have a similar safety profile to the invertases that were the subjects of GRN 000088, GRN 000537 and GRN 001006. Additionally, AB Enzymes states that a literature search did not identify any information that would contradict a general recognition of safety of the invertase enzyme preparation.

AB Enzymes discusses publicly available literature to address potential allergenicity due to invertase. Based on bioinformatic analyses, using criteria recommended by FAO/WHO (FAO/WHO, 2001; Codex Alimentarius, 2003), AB Enzymes reports that no sequence homology of *A. niger* invertase 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 invertase will result in allergenic responses from its intended uses.

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

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 invertase 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 invertase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing invertase enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

[3] The subject of GRN 000088 includes invertase enzyme preparation from *Saccharomyces cerevisiae*; the subject of GRN 000537 is sc-FOS; and the subject of GRN 001006 is sc-FOS. We evaluated these notices and responded in letters dated April 3, 2002, February 6, 2015, and January 19, 2022, respectively, stating that we had no questions at that time regarding the notifiers' GRAS conclusions.

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 invertase enzyme preparation is GRAS under its intended conditions of use. This letter is not an affirmation that invertase 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 001173 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2024.08.15 15:22:14 -04'00'

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