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

Re: GRAS Notice No. GRN 000982

Dear Mr. Trujillo:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000982. We received AB Enzymes GmbH (AB Enzymes)'s GRAS notice on November 16, 2020 and filed it on March 22, 2021. We received an amendment on November 19, 2021 clarifying information regarding the identity, genetic modification, manufacturing, and safety of the enzyme preparation.

The subject of the notice is polygalacturonase enzyme preparation produced by *Aspergillus oryzae* expressing a polygalacturonase gene from *A. tubingensis Mosseray* (polygalacturonase enzyme preparation) for use as an enzyme at up to 2 mg Total Organic Solids (TOS) per kg raw material in the processing of coffee, fruit and vegetable juices, flavoring substances, and wine. The notice informs us of AB Enzymes' view that this use of polygalacturonase 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 polygalacturonase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, polygalacturonase is identified by the Enzyme Commission Number 3.2.1.15.[1] AB Enzymes states that the polygalacturonase enzyme contains 368 residues and has a calculated molecular weight of 38.1 kDa.

AB Enzymes states that the *A. oryzae* production organism is non-pathogenic and non-toxigenic. AB Enzymes states that the recipient strain used in the construction of the

[1] https://iubmb.qmul.ac.uk/enzyme/EC3/2/1/15.html

production strain, AR-183, was genetically modified to overproduce polygalacturonase.[2] The production strain was constructed by first creating a spontaneous mutant recipient strain, followed by the insertion of an expression cassette carrying the *A. tubingensis Mosseray* polygalacturonase gene using a *A. oryzae* promoter, an *A. tubingensis* native terminator, and the *A. nidulans* acetamidase gene as a selection marker. AB Enzymes states it confirmed the insertion and absence of the vector backbone by Southern blot hybridization and DNA sequencing. AB Enzymes evaluated the stability of the integration by monitoring the growth during large-scale fermentation and production of the polygalacturonase enzyme. AB Enzymes states that the transformed DNA does not contain any antibiotic resistance genes. AB confirmed absence of antibiotic or toxic compounds in the final enzyme concentrate based on testing of three production batches.

AB Enzymes states that the polygalacturonase enzyme preparation is manufactured by fed-batch submerged fermentation of a pure culture of the *A. oryzae* AR-183 production strain under controlled conditions, and that the enzyme is secreted into the fermentation medium. AB Enzymes states that the enzyme is separated from the biomass by filtration or centrifugation, and then concentrated prior to polish filtration and germ filtration to remove the production organism. The resulting enzyme concentrate is preserved and formulated with sodium chloride, glycerol, and water to yield a liquid enzyme preparation. AB states that the enzyme concentrate prior to formulation is used for the safety studies discussed in the notice. AB Enzymes states that the entire process is performed in accordance with current good manufacturing practices using food grade raw materials. AB Enzymes also states that the fermentation medium used in the manufacturing of polygalacturonase enzyme preparation contains a wheat-based ingredient. AB Enzymes states that based on results from routine allergen risk assessment during the manufacturing of the polygalacturonase enzyme preparation and testing for trace gluten using the R5 antibody-based ELISA, the presence of gluten in the enzyme preparation is not a concern.[3]

AB Enzymes has established food-grade specifications and states that the polygalacturonase enzyme preparation conforms to specifications established for enzyme preparations in the 12th edition of the Food Chemicals Codex (FCC, 2020), 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 polygalacturonase enzyme concentrate to demonstrate that the manufacturing acceptance criteria have been met and that the production organism is absent from the final enzyme preparation.

AB Enzymes intends to use polygalacturonase enzyme preparation at a maximum use level of 2 mg TOS per kg raw material in the processing of fruits, vegetables, coffee,

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[2] AB Enzymes states that the recipient *A. oryzae* strain was produced by spontaneous mutation (nitrogen reductase mutant able to utilize nitrate as a nitrogen source) of the parental strain *A. oryzae* (Ahlburg) Cohn, which is deposited in the Westerdijk Fungal Biodiversity Institute in the Netherlands with the accession number CBS 146745.

[3] Results of analysis are below the limit of quantification for this assay (<5 mg/kg).

wine, and flavoring substances. The enzyme is added to the respective raw materials to break down pectin in fruit and vegetable purees for juice production, demucilation of coffee berry in coffee bean production, production of flavors from plant materials, and during multiple stages of winemaking. AB Enzymes states that no enzyme activity is expected to be present in the final food product, since the enzyme is either heat inactivated and/or removed by filtration during later stages of processing. AB Enzymes estimates a maximum dietary exposure to polygalacturonase enzyme preparation to be 0.105 mg TOS/kg body weight per day (mg TOS/kg bw/d) from the intended uses, and with the assumption that all of it will remain in the final food.[4]

AB Enzymes relies on published information that discusses the safety of the *A. oryzae* production organism and the safety of microbial enzyme preparations used in food processing. AB Enzymes summarizes corroborative unpublished toxicological studies using the polygalacturonase enzyme concentrate. Tests conducted with bacterial cells showed that the polygalacturonase is not mutagenic at the highest dose tested both in the presence and absence of metabolic activation. AB Enzymes also demonstrates that the polygalacturonase enzyme concentrate is not clastogenic based on results from *in vitro* mammalian cell micronucleus test. An unpublished 90-day oral toxicity study by AB Enzymes, using the polygalacturonase enzyme concentrate up to the highest dose tested (1000 mg TOS/kg bw/d), showed no treatment related effects in rats. Based on results from this study and the estimated dietary exposure from the intended uses of the polygalacturonase enzyme preparation (0.105 mg TOS/kg bw/d), AB Enzymes calculates the margin of exposure to be approximately 9500.[5]

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 uses. Based on bioinformatic 80-mer sliding window analyses using publicly available allergen protein databases, AB Enzymes states that the polygalacturonase has >35% sequence identity to pollen allergens of maize, subtropical Bahia grass, Japanese cedar pollen, and conifer *Cryptomeria japonica*. However, based on the totality of the information available, including fate of the polygalacturonase in the finished food, its bioinformatics, the available literature on its allergenic potential, and the reduced risk of allergenicity due to the pH of the gastrointestinal tract, AB Enzymes concludes that it is unlikely that oral consumption of polygalacturonase from the intended use will result in allergenic responses.

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

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[4] AB Enzymes uses the Budget method to estimate dietary exposure to polygalacturonase enzyme preparation based on a maximum use levels of 3.2 mg TOS/kg in liquid foods and 2 mg TOS/kg in solid foods respectively, and consumption of 25 mL of beverages and 12.5 g of solid foods per kg body weight per day.

[5] FDA notes the margin of exposure 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 polygalacturonase 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 polygalacturonase erase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing polygalacturonase 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 polygalacturonase enzyme preparation produced by *A. oryzae* expressing the gene encoding polygalacturonase from *A. tubingensis Mosseray* is GRAS under its intended conditions of use. This letter is not an affirmation that polygalacturonase enzyme preparation produced by *A. oryzae* expressing the gene encoding polygalacturonase from *A. tubingensis Mosseray* 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 000982 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

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