



Candice Cryne
AB Enzymes Inc.
8211 W. Broward Blvd. Suite 375
Plantation, FL 33324

Re: GRAS Notice No. GRN 000979

Dear Ms. Cryne:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000979. We received AB Enzymes' GRAS notice on November 16, 2020 and filed it on February 9, 2021. We received an amendment on August 15, 2021 clarifying the non-toxicity and construction of the production strain, the manufacturing, and the safety information of the pectin esterase enzyme.

The subject of the notice is pectin esterase enzyme preparation produced by *Aspergillus oryzae* expressing a gene encoding a pectin esterase gene from *A. tubingensis* (pectin esterase enzyme preparation) for use as an enzyme at up to 26 mg Total Organic Solids (TOS) per kg raw material, in the processing of fruits, vegetables, coffee, wine, and in the production of flavors. The notice informs us of AB Enzymes' view that this use of pectin 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 pectin esterase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, pectin esterase is identified the Enzyme Commission Number 3.1.1.11.¹ AB Enzymes states that the pectin esterase enzyme protein contains 314 residues and has a calculated molecular weight of 34 kDa.

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

¹ <https://www.qmul.ac.uk/sbcs/iubmb/enzyme/EC3/1/1/11.html>

production strain, AR-962, was genetically modified to overproduce pectin esterase.² The production strain was constructed by the integration of an expression cassette carrying the *A. tubingensis* pectin methylesterase gene and a selection cassette.³ AB Enzymes states it confirmed the insertion and absence of the vector backbone by Southern blot hybridization, DNA sequencing, Western blot, and SDS-PAGE analyses. AB Enzymes evaluated the stability of the integration over five generations by monitoring the growth during large-scale fermentation and production of the pectin esterase enzyme. AB Enzymes also verified the absence of any functional antibiotic resistance genes in the final production strain genome by Southern blot analysis.

AB Enzymes states that the pectin esterase enzyme preparation is manufactured by submerged fermentation of a pure culture of the *A. oryzae* AR-962 production strain under controlled conditions, and that the enzyme is secreted into the fermentation medium. AB Enzymes states that the enzyme is recovered from the fermentation medium by filtration or centrifugation, and then concentrated. After 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 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 pectin esterase enzyme preparation contains a wheat-based ingredient; however, absence of wheat protein is confirmed by using routine R5 antibody-based ELISA analysis.

AB Enzymes has established food-grade specifications and states that the pectin esterase 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 pectin esterase 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 pectin esterase enzyme preparation at a maximum use level of 26 mg Total Organic Solids (TOS) per kg raw material, in the processing of fruits, vegetables, coffee, wine, and in the production of flavors. AB Enzymes states that no enzyme activity is expected to be present in the final food product, since the enzyme is either heat inactivated or removed during processing. AB Enzymes estimates a maximum dietary exposure to pectin esterase enzyme preparation to be 0.39 mg TOS/kg body weight per day (mg TOS/kg bw/d) from the intended uses, and with the

² AB Enzymes states that the recipient *A. oryzae* strain was produced by spontaneous mutation 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 AB Enzyme states that transformation was performed with two plasmids, one carrying an expression cassette containing the *A. tubingensis* pectin methylesterase gene under the control of an *A. oryzae* promoter and the *amdS* selection marker gene from *A. nidulans*, and another carrying a selection cassette containing the nitrate reductase selection marker gene.

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 pectin esterase liquid enzyme concentrate. Tests conducted with bacterial cells showed that the pectin esterase is not mutagenic at the highest dose tested both in the presence and absence of metabolic activation. AB Enzymes also demonstrates that the pectin esterase enzyme concentrate is not clastogenic based on results from in vitro mammalian cell micronucleus test. A 90-day oral toxicity study in rats using the pectin esterase liquid enzyme concentrate at the highest dose tested (1000 mg TOS/kg bw/d) showed no treatment related effects. Based on the highest dose tested in the unpublished 90-day study (1000 mg TOS/kg bw/d) and the estimated dietary exposure from the intended uses of the pectin esterase enzyme preparation (0.39 mg TOS/kg bw/d), AB Enzymes calculates the margin of safety to be approximately 2600.⁵

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 analyses using FARRP allergen protein database, AB Enzymes reports that the notified pectin esterase shares >35% sequence identity with pollen allergens of a common weed *Salsola kali* and olive tree *Olea europaea* across a window of 80 amino acids. Based on the totality of the information available, including fate of the pectin esterase in final food, its bioinformatics, literature on allergenic potential, and the pH of the gastrointestinal tract, AB Enzymes concludes that it is unlikely that oral consumption of pectin esterase from the intended use will result in allergenic responses.

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

Section 301(II) of the Federal Food, Drug, and Cosmetic Act (FD&C Act)

Section 301(II) 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(II)(1)-(4) applies. In our evaluation of AB Enzymes' notice concluding that pectin esterase enzyme preparation is GRAS under its intended conditions of use, we did not consider whether section 301(II) or any of its exemptions apply to foods containing pectin esterase enzyme preparation. Accordingly, our response should not be

⁴ AB Enzymes uses the Budget method to estimate dietary exposure to pectin esterase enzyme preparation based on a maximum use levels of 2.6 mg TOS/kg in liquid foods and 26 mg TOS/kg in solid foods respectively, and consumption of a maximum 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.

construed to be a statement that foods containing pectin esterase enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(l).


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 pectin esterase enzyme preparation produced by *A. oryzae* expressing the gene encoding pectin esterase from *A. tubingensis* is GRAS under its intended conditions of use. This letter is not an affirmation that pectin esterase enzyme preparation produced by *Aspergillus oryzae* expressing the gene encoding pectin esterase from *Aspergillus tubingensis* 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 000979 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J.
Carlson -S

 Digitally signed by Susan J. Carlson -S
Date: 2021.10.18 17:36:40 -04'00'

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