Madhu Soni
Soni & Associates Inc.
749 46th Square
Vero Beach, FL 32968

Re: GRAS Notice No. GRN 001097

Dear Dr. Soni,

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001097. We received the notice that you submitted on behalf of Advanced Enzymes Technologies Ltd.'s (Advanced Enzymes) on July 6, 2022, and filed it on February 14, 2023. Advanced Enzymes submitted amendments to the notice on September 25, 2023, and October 24, 2023, that provided clarifications on the identity, specifications, dietary exposure, and safety narrative.

The subject of the notice is maltogenic alpha-amylase enzyme preparation produced by *Escherichia coli* expressing the maltogenic alpha-amylase from *Geobacillus stearothermophilus* (maltogenic alpha-amylase enzyme preparation) for use as an enzyme at up to 34 mg total organic solids (TOS)/kg raw material in baking, brewing, and starch processing. The notice informs us of Advanced Enzymes' view that this use of maltogenic alpha-amylase 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. Advanced Enzymes' notice provides information about the components in the maltogenic alpha-amylase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, maltogenic alpha-amylase is identified by the Chemical Abstracts Service number 160611-47-2 and the Enzyme Commission Number 3.2.1.133.[1] Advanced Enzymes states that the primary sequence of maltogenic alpha-amylase consists of 686 amino acids with a calculated molecular weight of 75.2 kDa.

Advanced Enzymes states that the *E. coli* production organism is non-pathogenic and non-toxigenic and is a well-characterized production organism with history of safe use

[1] https://iubmb.qmul.ac.uk/enzyme/EC3/2/1/133.html

in the food industry. Advanced Enzymes states that the production strain, "BLASC," was constructed from the parent strain, *E. coli* BL21(DE3), through transformation with a plasmid carrying the maltogenic alpha-amylase gene from *G. stearothermophilus* and regulatory elements, as well as a post-segregational killing gene.[2] Advanced Enzymes states that they confirmed sequence integrity by polymerase chain reaction (PCR) and genetic sequencing. Advanced Enzymes evaluated the genetic stability of the production strain using random amplified polymorphic DNA fingerprinting, in conjunction with the post-segregational killing system. Advanced Enzymes verified the absence of functional or transferable antibiotic resistance genes in the final production strain genome.

Advanced Enzymes states that the maltogenic alpha-amylase enzyme preparation is produced by submerged fermentation of a pure culture of the *E. coli* production strain under controlled conditions. The maltogenic alpha-amylase enzyme is produced intracellularly and then released into the fermentation medium using heat and homogenization. The released enzyme is then recovered by flocculation and filtration. This is followed by concentration and centrifugation. The resulting liquid concentrate is spray-dried and stabilized with food-grade stabilizers, such as maltodextrin. The final enzyme concentrate is a light brown to brown powder. Advanced Enzymes states that the fermentation medium does not contain any major allergens or components derived from allergenic sources. Advanced Enzymes states that the entire process is performed using food grade raw materials and in accordance with current good manufacturing practices.

Advanced Enzymes has established food grade specifications and states that the maltogenic alpha-amylase 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). Advanced Enzymes provides results from analyses of three non-consecutive batches of maltogenic alpha-amylase enzyme concentrate to demonstrate that the manufacturing acceptance criteria can be met, including the absence of the production organism and antibiotic activity.

Advanced Enzymes intends to use maltogenic alpha-amylase enzyme preparation at a maximum use level of 34 mg TOS/kg raw material in baking, brewing, and starch processing. Advanced Enzymes states that maltogenic alpha-amylase hydrolyzes (1-4)-alpha-D-glucosidic linkages in starch polysaccharides to remove successive alpha-maltose residues from the non-reducing ends of the chains. Advanced Enzymes notes that the final enzyme is inactivated or denatured during processing. Advanced Enzymes estimates a maximum dietary exposure to maltogenic alpha-amylase enzyme preparation to be 0.92 mg TOS/kg body weight per day (mg TOS/kg bw/d) from the intended uses and assumes that all of the maltogenic alpha-amylase enzyme preparation

[2] Advanced Enzymes states that the production strain contains a post-segregational killing system for cell selection, which is comprised of two genes, *ccd*B and *ccd*A. The gene *ccd*B is integrated with the host genome, while the gene *ccd*A is present on the plasmid. The segregation (loss) of the plasmid from the cells results in cell death due to the expression of *ccd*B gene, which is otherwise ineffective due to the presence of *ccd*A.

will be active and remain in the final food.[3]

Advanced Enzymes relies on published information that discusses the safety of the *E. coli* production organism, including prior use of the parent strain for production of food ingredients, and the safety of microbial enzyme preparations used in food processing. Advanced Enzymes states that the maltogenic alpha-amylase enzyme preparation did not show sequence homology with known toxin protein sequences. Additionally, Advanced Enzymes states that a literature search did not identify any information that would contradict a general recognition of safety of the maltogenic alpha-amylase enzyme preparation. Advanced Enzymes summarizes unpublished toxicological studies using the maltogenic alpha-amylase enzyme preparation to corroborate their safety conclusion. Advanced Enzymes states that maltogenic alpha-amylase is not mutagenic based on the results of the bacterial reverse mutation assay and an *in vitro* chromosomal aberration test in human lymphocytes. In a 90-day repeated-dose oral toxicity study, rats were given the maltogenic alpha-amylase enzyme preparation, and no treatment-induced adverse effects were noted up to the highest dose tested (equivalent to 838 mg TOS/kg bw). Advanced Enzymes calculated the margin of safety to be 913.[4]

Advanced Enzymes discusses publicly available literature to address potential allergenicity due to maltogenic alpha-amylase. Based on bioinformatic analysis, Advanced Enzymes reports no significant matches between the amino acid sequences of the maltogenic alpha-amylase and the primary sequences of known food allergens based on the guidelines developed by the FAO/WHO in 2001 (Food and Agriculture Organization of the United Nations, January 2001). Advanced Enzymes further states that any active or inactive maltogenic alpha-amylase remaining in the final food will be digested in the gastrointestinal system of the consumer into small peptides and amino acids, further reducing the risk of potential allergenicity. Based on the totality of information available, Advanced Enzymes concludes that it is unlikely that oral consumption of maltogenic alpha-amylase preparation from the intended uses will result in allergic responses.

Based on the data and information summarized above, Advanced Enzymes concludes that maltogenic alpha-amylase enzyme preparation is GRAS for its intended use.

Standards of Identity

In the notice, Advanced Enzymes states its intention to use maltogenic alpha-amylase 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

[3] Advanced Enzymes uses the Budget method to estimate the dietary exposure to maltogenic alpha-amylase enzyme preparation based on the consumption of 12.5 g baked goods per kg body weight per day and 25 mL starch-based beverages per kg body weight per day (worst case scenario) containing the maltogenic alpha-amylase enzyme preparation at the maximum use level.

[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.

added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity.

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 Advanced Enzymes' notice concluding that maltogenic alpha-amylase 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 maltogenic alpha-amylase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing maltogenic alpha-amylase enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Advanced Enzymes provided, as well as other information available to FDA, we have no questions at this time regarding Advanced Enzymes' conclusion that maltogenic alpha-amylase enzyme preparation is GRAS under its intended conditions of use. This letter is not an affirmation that maltogenic alpha-amylase 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 001097 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2023.12.07 19:03:50 -05'00'

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