



Candice Cryne
AB Enzymes GmbH
Feldbergstr. 78
D-64293 Darmstadt, GERMANY

Re: GRAS Notice No. GRN 000707

Dear Ms. Cryne:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000707. We received AB Enzymes GmbH (AB Enzymes)'s GRAS notice on May 16, 2017 and filed it on June 22, 2017. We received amendments containing additional safety information on June 22, 2017, and October 27, 2017.

The subject of the notice is glucose oxidase enzyme preparation produced by *Trichoderma reesei* expressing a modified synthetic gene encoding glucose oxidase from *Penicillium amagasakiense* (glucose oxidase enzyme preparation) for use as an enzyme in the production of baked goods and cereal-based products at a maximum level of 10 mg Total Organic Solids (TOS)/kg flour. The notice informs us of AB Enzymes' view that these uses of glucose oxidase enzyme preparation are 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 glucose oxidase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, glucose oxidase is identified by the Enzyme Commission Number 1.1.3.4. The accepted name and systematic name is glucose oxidase. This enzyme is also known as β -D-glucose oxidase, β -D-glucose: quinone oxidoreductase, D-glucose oxidase, D-glucose-1-oxidase, glucose oxyhydrase, deoxin-1, glucose aerodehydrogenase, aero-glucose dehydrogenase, glucose oxyhydrase, notatin, corylophyline, and penatin. Glucose oxidase catalyzes the oxidation of β -D-glucose to D-glucono-1,5-lactone with the concomitant reduction of molecular oxygen to hydrogen peroxide. The CAS Number for glucose oxidase is 9001-37-0. AB Enzymes states that the primary amino acid sequence of the expressed glucose oxidase has been verified to be 587 amino acids. AB Enzymes states that glucose oxidase has a molecular weight of 64 kDa. AB Enzymes confirmed the identity of the enzyme by N-terminal sequencing and mass spectrometry.

U.S. Food & Drug Administration
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AB Enzymes states that the *T. reesei* production strain was constructed from the recipient strain *T. reesei* RF10310.¹ AB Enzymes describes *T. reesei* as a non-pathogenic, non-toxicogenic, well-characterized production organism with a history of safe use in the food industry. AB Enzymes also states that the production strain is considered suitable for Good Industrial Large Scale Practice worldwide.

AB Enzymes describes the construction of the production strain *T. reesei* RF11400² from RF10310 by the targeted integration of an expression cassette carrying a modified synthetic gene encoding a variant of the wild-type glucose oxidase gene from *P. amagasakiense*,³ a modified *T. reesei* promoter, a native *T. reesei* transcriptional terminator, as well as an acetamidase selectable marker from *A. nidulans*. AB Enzymes confirmed the sequences of the inserted expression cassettes and the flanking regions at integration loci. AB Enzymes also confirmed that the introduced DNA is stable during production, via Southern blot hybridization, and is free of any functional antibiotic resistance genes.

AB Enzymes states that glucose oxidase enzyme is produced by submerged fed-batch fermentation of a pure culture of the production strain. AB Enzymes states that fermentation is carried out under controlled conditions and that the enzyme is secreted into the culture medium. After fermentation, the culture medium is pretreated with the addition of flocculants, water or filter aids. The enzyme is then recovered from the culture medium by filtration or centrifugation and then concentrated by ultracentrifugation. The concentrated enzyme solution is then filtered to ensure the removal of any production organism. The enzyme concentrate is formulated to an enzyme preparation with sunflower oil and wheat flour. 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 final enzyme preparation does not contain any major food allergens from the culture medium.

AB Enzymes states that the glucose oxidase enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 10th edition, 2016, 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 analytical data from one batch of glucose oxidase enzyme to demonstrate consistency with the specifications. AB Enzymes also confirms that a test for absence of any production organism in the final product is an established specification.

AB Enzymes intends to use glucose oxidase enzyme preparation to remove glucose or oxygen during edible oil refining process. The maximum level of glucose oxidase enzyme preparation for the intended use corresponds to 10 mg TOS/kg of flour. AB Enzymes notes that the glucose oxidase enzyme preparation will be denatured or removed during

¹ AB Enzymes states the recipient strain RF10310 was derived from *T. reesei* RH4847, which has been deposited in Centraalbureau voor Schimmelcultures (CBS) in the Netherlands as CBS114041.

² AB Enzymes states the production strain *T. reesei* RF11400 was deposited in CBS as CBS138879.

³ AB Enzymes states that the glucose oxidase gene is a synthetic gene that was codon optimized for expression in *T. reesei*.

baking. To estimate dietary exposure to glucose oxidase enzyme preparation, AB Enzymes assumes that the glucose oxidase enzyme preparation will be used at the maximum intended levels, and that the enzyme preparation will remain in the final food. Based on these assumptions, AB Enzymes estimates a maximum dietary exposure of glucose oxidase enzyme preparation from all intended uses to be 0.088 mg TOS/kg body weight per day (mg TOS/kg bw/d). AB Enzymes states that the reaction products of the catalytic activity of glucose oxidase would be considered normal constituents of the diet, and no adverse effect on nutrients is expected.

AB Enzymes relies on published information that discusses the safety of microbial enzyme preparations used in food processing, including the safety of the production organism. Additionally, to corroborate safety of the intended uses, AB Enzymes summarizes unpublished toxicological studies using the glucose oxidase enzyme liquid concentrate. AB Enzymes states that the glucose oxidase enzyme is not mutagenic based on results from a bacterial reverse mutation assay, and on results from an *in vitro* micronucleus assay in cultured human lymphocytes. A 13-week/90-day oral toxicity study in rats using the glucose oxidase enzyme concentrate did not cause any treatment-related adverse effects up to the highest dose tested (equivalent to 1000 mg TOS/kg bw/d). Based on the highest dose tested in the 13-week study and the estimated dietary exposure from the intended uses of the glucose oxidase enzyme preparation, AB Enzymes calculates a margin of safety to be 11,429. FDA notes the margin of safety is based on unpublished safety studies, and is corroborative of the published information regarding enzyme preparations used in food processing.

AB Enzymes discusses potential food allergenicity and toxicity of glucose oxidase enzyme. AB Enzymes states that naturally occurring food enzymes, if present in the final food, are unlikely to have allergenic potential because they are present in low concentrations, are denatured during processing, and are susceptible to digestion in the gastrointestinal system. Additionally, AB Enzymes conducted bioinformatic analyses of the glucose oxidase enzyme sequence for >35% homology in a sliding window of 80 amino acids and of eight amino acids against known allergens in the Food Allergy Research and Resource Program allergen protein database. The 80-mer sliding window analysis found a 41.2% identity match with Mala s 12, an allergen from the fungal species *Malassezia sympodialis*. AB Enzymes states that the homology match is likely due to sequence conservation among functionally related oxidoreductases, which include glucose oxidase. AB Enzymes did not find any significant homology between sequences of eight contiguous amino acids of glucose oxidase and known allergenic proteins. AB Enzymes states that glucose oxidase was included in a published study of the allergenic potential of industrial food enzymes for 400 allergic patients and found to be of no concern if ingested as food. AB Enzymes also assessed the sequence homology of glucose oxidase to known toxins in the non-redundant protein sequences database using the BLAST-P (protein – protein BLAST) database and did not identify any significant homology to any protein sequence identified or known to be a toxin. AB Enzymes further cites the conclusions of several organizations and working groups about the low risk of allergenicity posed by enzymes due to their low use levels and the extensive processing of enzyme-containing foods during manufacturing. Based on the

totality of the information available, AB Enzymes concludes that it is unlikely that oral consumption of glucose oxidase enzyme will result in any allergenic responses.

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

Standards of Identity

In the notice, AB Enzymes states its intention to use glucose oxidase enzyme preparation in several food categories, including foods for which standards of identity exist, located in Title 21 of the Code of Federal Regulations. 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 eight foods or food groups (i.e., milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, and soybeans) or a food ingredient that contains protein derived from one of those foods. Glucose oxidase enzyme preparation requires labeling under the FD&C Act because it contains protein derived from wheat.

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


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 glucose oxidase enzyme preparation produced by *T. reesei* expressing a modified synthetic gene encoding glucose oxidase from *P. amagasakiense* is GRAS under its intended conditions of use. This letter is not an affirmation that glucose oxidase enzyme

preparation produced by *T. reesei* expressing a modified synthetic gene encoding glucose oxidase from *P. amagasakiense* 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 000707 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,
**Michael A.
Adams -S**
Dennis M. Keefe, Ph.D.
Director
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition



Digitally signed by Michael A. Adams -S
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