



Gary L. Yingling
Morgan, Lewis & Bockius LLP
1111 Pennsylvania Avenue, NW
Washington, DC 20004

Re: GRAS Notice No. GRN 000750

Dear Mr. Yingling:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000750. We received the notice that you submitted on behalf of DSM Food Specialties (DSM) on December 18, 2017 and filed it on January 8, 2018. We received amendments on June 1, 2018, and September 19, 2018, containing additional safety information and clarification on information initially designated as confidential.¹ Further, in the amendment dated September 19, 2018, DSM states that the beta-glucosidase is an additional enzyme activity contained in the pectinase enzyme preparation that was one of the subjects of GRN 000089.²

The subject of the notice is beta-glucosidase enzyme preparation produced by *Aspergillus niger* (beta-glucosidase enzyme preparation) for use as an enzyme up to 363 mg Total Organic Solids (TOS)/kg wort, in the production of fermented beverages. The notice informs us of DSM's view that this use of beta-glucosidase 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. DSM's notice provides information about the components in the beta-glucosidase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, beta-glucosidase is identified by the Enzyme Commission Number 3.2.1.21. The accepted name is beta-glucosidase and systematic name for this enzyme is beta-D-glucoside glucohydrolase. The enzyme is also

¹ GRN 000750 included information in Annexes 7, 8, 9, and 10 that DSM initially designated confidential. In the June 1, 2018, amendment, DSM confirms that the information was incorrectly marked confidential and that this information is not confidential.

² GRN 000089 describes the use of enzyme preparations, including pectinase enzyme preparation, from *A. niger* for use as enzymes in food. FDA evaluated this notice and responded with a letter dated April 3, 2002, stating that we had no questions at that time regarding Enzyme Technical Association's GRAS conclusion, which was based on common use in food.

U.S. Food & Drug Administration
Center for Food Safety & Applied Nutrition
5001 Campus Drive
College Park, MD 20740

known as gentiobiase, cellobiase, emulsin, elaterase, aryl- β -glucosidase, β -D-glucosidase, β -glucoside glucohydrolase, arbutinase, amygdalinase, p-nitrophenyl B-glucosidase, primeverosidase, amygdalase, linamarase, salicilinase, and β -1,6-glucosidase. Beta-glucosidase hydrolyzes the O-glycosyl bond between the terminal (non-reducing) glucose residue and the rest of the molecule in glycosides. The CAS No. for beta-glucosidase is 160611-47-2. DSM provides the amino acid sequence for beta-glucosidase.

DSM describes *A. niger* as a non-pathogenic, non-toxicogenic, and well-characterized production organism with a history of safe use in the food industry. DSM states that the *A. niger* production strain ARO1 is a classical strain not subjected to genetic engineering for production of the beta-glucosidase enzyme. DSM states that the strain is genetically stable and has been maintained for more than 20 years.

DSM states that beta-glucosidase enzyme is produced by submerged fed-batch fermentation of a pure culture of the *A. niger* ARO1 strain, as described in GRN 000089 for production of pectinase. DSM states that fermentation is carried out under controlled conditions and that the beta-glucosidase is secreted into the culture medium. Beta-glucosidase is recovered from the culture medium by filtration or centrifugation of the supernatant and concentrated by ultrafiltration. DSM provides analytical data from three batches of beta-glucosidase enzyme concentrate to demonstrate manufacturing consistency. The liquid enzyme concentrate is standardized to a beta-glucosidase enzyme preparation by the addition of glycerol. DSM states that the production process is in accordance with current good manufacturing practices. DSM states that the beta-glucosidase 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). DSM also confirms that a test for absence of any production organism in the final product is an established specification. DSM states that the final enzyme preparation does not contain any major food allergens from the culture medium.

DSM intends to use beta-glucosidase enzyme preparation in the brewing of fermented beverages at a maximum level corresponding to 363 mg TOS/kg wort. DSM estimates dietary exposure from all uses of beta-glucosidase enzyme preparation to be 0.38 mg TOS/kg body weight per day (mg TOS/kg bw/d), based on 95th percentile estimates of beer consumption in the U. S.,³ and on the assumptions that the beta-glucosidase enzyme preparation will be used at the maximum intended levels, and that all of the enzyme preparation will remain in the final food.⁴

DSM relies on published information that discusses the safety of microbial enzyme preparations used in food processing, including the safety of the production organism.

³ DSM bases their estimation on data from Trends in Food and Nutrient Intakes by Adults: NFCS 1977–78, CSFI 1989–91, and CSFII 1994–95.

⁴ FDA estimates mean and 90th percentile dietary exposure to beta-glucosidase enzyme preparation from beer consumption to be 0.75 mg TOS/kg bw/d and 1.7 mg TOS/kg bw/d, based on 2003–2014 NHANES combined 2-day consumption data.

Additionally, DSM summarizes the results of unpublished toxicological studies performed using a liquid enzyme concentrate, containing both pectinase and beta-glucosidase, to corroborate safety of the intended uses of the beta-glucosidase enzyme preparation produced from the concentrate. A 13-week oral toxicity study in rats using the pectinase enzyme complex concentrate that contains beta-glucosidase did not reveal any treatment-related adverse effects up to the highest dose tested (equivalent to 440 mg TOS/kg bw/d). Based on the highest dose tested in the 13-week study and their estimated dietary exposure from the intended uses of the beta-glucosidase enzyme preparation, DSM calculates a margin of exposure to be 1164. 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.

DSM discusses potential food allergenicity of beta-glucosidase enzyme. DSM 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 and are susceptible to digestion in the gastrointestinal system. DSM conducted a sequence homology search with a window of 80 amino acids from the peptide sequence of beta-glucosidase against known allergens stored in the AllergenOnline database. DSM reports that two stretches of amino acid sequences above a 35% threshold are homologous to the protein Asp n 14 from *A. niger*, which is not considered an oral allergen.⁵ Additionally, DSM conducted a search for 100% identity over 8 contiguous amino acids that did not produce any hits to known allergens. DSM 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, DSM concludes that it is unlikely that oral consumption of beta-glucosidase enzyme will result in any allergenic responses.

Based on the data and information summarized above, DSM concludes that beta-glucosidase 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 DSM's notice concluding that beta-glucosidase 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 beta-glucosidase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing beta-glucosidase enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

⁵ Asp n 14 (beta-xylosidase) is an occupational allergen associated with baker's asthma.

Conclusions

Based on the information that DSM provided, as well as other information available to FDA, we have no questions at this time regarding DSM's conclusion that beta-glucosidase enzyme preparation produced by *A. niger* is GRAS under its intended conditions of use. This letter is not an affirmation that beta-glucosidase enzyme preparation produced by *A. niger* 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 000750 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.
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