Katherine Vega, Ph.D.
DSM Food Specialties B.V.
P.O. Box 1
2600 MA Delft
Netherlands

Re: GRAS Notice No. GRN 000832

Dear Dr. Vega:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000832. We received DSM Food Specialties B.V.'s (DSM) notice on January 28, 2019 and filed on March 13, 2019. In amendments dated August 23, 2019, August 30,2019, and October 18, 2019, you clarified details regarding identity, manufacturing, specifications, and classification.

The subject of the notice is acid prolyl endopeptidase enzyme preparation produced by *Aspergillus niger* overexpressing native acid prolyl endopeptidase (acid prolyl endopeptidase enzyme preparation) for use as an enzyme at a maximum use level of 6.3 mg Total Organic Solids (TOS)/kg raw material in the production of fermented beverages. The notice informs us of DSM's view that this use of acid prolyl endopeptidase 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 acid prolyl endopeptidase enzyme preparation.

According to the notifier, acid prolyl endopeptidase is currently not classified by the Enzyme Commission; however, DSM states that native acid prolyl endopeptidase has similar activity to EC Number 3.4.21.26.[1] DSM states that the molecular weight of the acid prolyl endopeptidase is 56 kDa and provides the amino acid sequence.

DSM describes the construction of the non-pathogenic and non-toxigenic *A. niger* GEP production strain from *A. niger* DS 38556; a genetically modified strain derived from *A. niger* NRRL 3122 strain. Specifically, DSM inserted multiple expression cassettes containing the acid prolyl endopeptidase *gep*A gene *via* targeted integration into (seven)

[1] https://www.qmul.ac.uk/sbcs/iubmb/enzyme/EC3/4/21/26.html

endogenous loci. DSM used Southern blot and PCR analyses to verify the integration of twenty copies of the acid prolyl endopeptidase gene as well as the deletion of the glucoamylase and protease genes. DSM states that the final production strain does not contain any selection markers or heterologous DNA.

DSM states that acid prolyl endopeptidase enzyme preparation is manufactured by controlled submerged fermentation of a pure culture of the *A. niger* GEP production strain. DSM notes that the fermentation medium contains a wheat-derived glucose syrup that is refined to remove proteins.[2] The secreted enzyme is recovered by a series of filtration and concentration steps, and then formulated with glycerol into the commercial preparation. DSM states that the concentrated enzyme, prior to formulation, is used for the safety studies discussed in this notice. DSM states that the entire process is performed using food-grade raw materials and in accordance with current good manufacturing practices.

DSM has established food grade specifications and states that the acid prolyl endopeptidase enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 11th edition, 2018), 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 provides analytical data from three batches of acid prolyl endopeptidase enzyme concentrate to demonstrate that the manufacturing acceptance criteria can been met.

DSM intends to use acid prolyl endopeptidase enzyme preparation at a maximum use level of 6.3 mg TOS/kg raw material in the production of fermented beverages. DSM notes that the acid prolyl endopeptidase enzyme preparation will be deactivated or removed during production or refining. However, in estimating dietary exposure, DSM assumes that all the acid prolyl endopeptidase enzyme preparation will remain in the final food. DSM estimates dietary exposure to acid prolyl endopeptidase enzyme preparation to be up to 0.6 mg TOS/kg body weight per day (mg TOS/kg bw/d) from all the intended uses.[3]

DSM relies on published information on the safety of the *A. niger* production organism and the safety of microbial enzyme preparations used in food processing. DSM discusses an unpublished bacterial reverse mutation test and an *in vitro* chromosomal aberration test in human lymphocytes to support their conclusion that the subject of the notice is neither genotoxic nor clastogenic. DSM also discusses the results from an unpublished 90-day oral (gavage) toxicity study of the acid prolyl endopeptidase enzyme concentrate in rats. DSM states no treatment-related adverse effects at the highest dose of acid prolyl

[2] DSM states that the absence of protein in the glucose syrup is verified by ELISA.
[3] DSM uses beer as a proxy in their consumer intake evaluation. DSM assumed consumption of up to 17 drinks per person per week and a body weight of 60 kg in their dietary exposure calculations. A standard beer is approximately 355 mL.

endopeptidase enzyme concentrate tested was observed; this is equivalent to 5040 mg TOS/kg bw/d.

DSM discusses publicly available literature, as well as the conclusions of several organizations and working groups, describing the low risk of allergenicity posed by enzymes to address potential allergenicity of acid prolyl endopeptidase. Further, based on bioinformatic analyses, DSM reports that the acid prolyl endopeptidase does not share any biologically meaningful sequence homology to potential oral allergens or toxins. Based on the totality of the information available, DSM concludes that it is unlikely that oral consumption of acid prolyl endopeptidase enzyme will result in any allergenic or toxic responses.

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

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 acid prolyl endopeptidase enzyme preparation produced by *Aspergillus niger* overexpressing the native acid prolyl endopeptidase gene is GRAS under its intended conditions of use. This letter is not an affirmation that is acid prolyl endopeptidase enzyme preparation produced by *Aspergillus niger* overexpressing the native acid prolyl endopeptidase gene 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)(1), the information in this notice described in 21 CFR 170.225(c)(2) through (c)(5) will be accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2019.12.09 14:26:30 -05'00'

Susan Carlson, Ph.D.
Director
Division of Food Ingredients
Office of Food Additive Safety
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