U.S. FOOD & DRUG ADMINISTRATION

Human Foods Program

Wing Yu
CIRS GROUP USA INC
4250 Fairfax Drive, Suite 600
Arlington, VA 22203

Re: GRAS Notice No. GRN 001207

Dear Ms. Yu:

This letter corrects our response letter to GRN 001207 signed on April 10, 2025. The purpose of this revised letter is to correct the description of the production organism in paragraph 5 of the original response letter. The original response letter referred to the production organism, *Aspergillus oryzae* 90402, as a "yeast," but the correct description of the production organism is a "fungus."

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001207. We received the notice that you submitted on behalf of Nanjing Bestzyme Bio-Engineering Co. Ltd. (Nanjing Bestzyme) on August 2, 2024, and filed it on September 25, 2024. Nanjing Bestzyme submitted amendments to the notice on December 19, 2024, February 16, 2025, and March 10, 2025, providing additional information about the sweetness intensity, raw materials, specifications, and safety of the notified substance.

The subject of the notice is brazzein preparation produced by *Aspergillus oryzae* 90402 expressing a gene encoding for brazzein from *Pentadiplandra brazzeana* (brazzein preparation) for use as a general-purpose sweetener in food at levels consistent with current good manufacturing practices.[1]

Our use of the terms, "brazzein preparation produced by *Aspergillus oryzae* 90402 expressing a gene encoding for brazzein from *Pentadiplandra brazzeana*" or "brazzein preparation" in this letter is not our recommendation of that term as an appropriate common or usual name for declaring the substance in accordance with FDA's labeling requirements. Under 21 CFR 101.4, each ingredient must be declared by its common or usual name. In addition, 21 CFR 102.5 outlines general principles to use when establishing common or usual names for nonstandardized foods. Issues associated with labeling and the common or usual name of a food ingredient are under the purview of the Office of Nutrition and Food Labeling (ONFL) in the Nutrition Center for Excellence. The Office of Pre-Market Additive Safety (OPMAS) did not consult with

[1] Nanjing Bestzyme states that brazzein preparation is not intended for use in infant formula, foods under the jurisdiction of the U.S. Department of Agriculture, or in foods in which standards of identity preclude its use.

ONFL regarding the appropriate common or usual name for "brazzein preparation."

Nanjing Bestzyme provides information about the identity and composition of brazzein preparation. Brazzein preparation is produced using a genetically engineered *A. oryzae* 90402 that expresses a gene encoding for brazzein. Nanjing Bestzyme states that brazzein preparation is a light yellow to brownish yellow powder containing ≥95% (on a dry basis) brazzein, small amounts of carbohydrates and ash, and a minor amount of residual *A. oryzae* proteins carried over from the fermentation process. Nanjing Bestzyme states that brazzein preparation contains the 53-amino-acid isoform of brazzein, naturally present in the fruit of *P. brazzeana*, known as oubli fruit.

Nanjing Bestzyme states that the production strain, *A. oryzae* 90402, is a non-pathogenic, non-toxigenic, and well-characterized fungus with a history of safe use in the food industry. Nanjing Bestzyme states that the production strain is derived from *A. oryzae* A1560 and is constructed through a series of transformations with different expression constructs to enable the production of brazzein. Nanjing Bestzyme states that *A. oryzae* 90402 does not contain any antibiotic resistance genes or plasmids and is not capable of transferring plasmids or antibiotic resistance to other organisms.

Nanjing Bestzyme states that brazzein preparation is manufactured through controlled fermentation of *A. oryzae* 90402. The fermentation broth containing secreted brazzein is diluted with water. Filter aids and flocculants are added and then the broth is subjected to solid-liquid separation to remove the microbial biomass. The resulting solution containing brazzein is pH-adjusted with citric acid to a pH of 4.0, sodium benzoate is added, and then the solution is subjected to nanofiltration to concentrate the protein. The concentrate is then subjected to membrane filtration to remove residual cells and insoluble materials and to produce a sterile protein solution. This concentrate undergoes further purification by cation exchange chromatography and subsequent nanofiltration to remove salts and residual heavy metals. The resulting solution is stabilized and standardized with water and pH regulators as required and filtered. The sterile solution is spray freeze dried to obtain the final product. Nanjing Bestzyme states that brazzein preparation is manufactured according to good manufacturing practices and that all raw materials, processing aids, and food contact substances used in the manufacturing process for brazzein preparation are used in accordance with applicable U.S. regulations, are GRAS for their intended uses, or are the subject of an effective food contact notification. Nanjing Bestzyme discusses that none of the raw materials used in the fermentation media are major food allergens, nor are they derived from major food allergens.

Nanjing Bestzyme provides specifications for brazzein that include total brazzein (≥95% on a dry basis), moisture (≤9%), total nitrogen (≥15% on a dry basis), carbohydrates (≤3% on a dry basis), sulfate ash (≤1% on a dry basis), and limits for heavy metals, including lead (≤0.2 mg/kg), and microorganisms. Nanjing Bestzyme provides analytical results from three non-consecutive batches[2] to demonstrate that brazzein preparation can be manufactured to meet the specifications. Nanjing Bestzyme states

[2] Nanjiing Bestzyme provides results from the analyses of 5 non-consecutive batches for lead and cadmium.

that the shelf life of brazzein preparation is at least two years.

Nanjing Bestzyme provides estimates of dietary exposure to brazzein preparation based on the relative sweetness intensity of the notified substance and the methodology presented in a published study (Renwick, 2008; Ref. 2). The published study reported average and upper percentile (i.e., 90th percentile and higher) estimates of dietary exposure to intense sweeteners among children and adults with and without diabetes and estimated the dietary exposure to a sweetener based on its relative sweetness to sucrose and an assumption of its substitutional use. Based on the methodology described in Renwick, 2008 and the estimated relative sweetness intensity of brazzein preparation (24,000 times sweeter than sucrose), Nanjing Bestzyme estimates the average and high percentile dietary exposures to brazzein preparation for non-diabetic adults (0.011 and 0.028 mg/kg body weight (bw)/d, respectively), diabetic adults (0.012 and 0.037 mg/kg bw/d, respectively), non-diabetic children (0.018 and 0.041 mg/kg bw/d, respectively), and diabetic children (0.028 and 0.038 mg/kg bw/day, respectively).

Nanjing Bestzyme discusses publicly available data and information supporting the safety of brazzein preparation and its production organism, *A. oryzae* 90402. Nanjing Bestzyme describes brazzein as the principal sweetening component of brazzein preparation and notes that it is similar to native brazzein protein found in the fruit of the West African *P. brazzeana* plant. Nanjing Bestzyme notes previous human consumption of *P. brazzeana* fruit as part of the diet in endemic regions of Africa, suggesting previous consumption of brazzein as a component of human food. Nanjing Bestzyme states that the subject brazzein preparation is similar to the subject of GRN 001142[3] and incorporates relevant safety information into the notice. Nanjing Bestzyme describes the biochemical mechanism of brazzein's effect on sweet taste perception and notes that the mechanism is similar to that of other known sweet proteins.

Nanjing Bestzyme summarizes the results of a comprehensive literature search through December 2024 to identify available safety information relevant to brazzein preparation produced by *A. oryzae* 90402 and does not identify any safety concerns or information that would contradict its GRAS conclusion. Nanjing Bestzyme provides a summary of a published 90-day subchronic oral toxicity study to support the safety of the intended use of brazzein preparation. Additionally, Nanjing Bestzyme describes additional corroborative safety information in the form of unpublished genotoxicity, repeat-dose oral toxicity, and teratogenicity studies of the subject brazzein preparation. Based on the weight of evidence, including the results of *in silico* sequence-alignment-based approaches, Nanjing Bestzyme concludes that brazzein does not pose an allergenic or toxigenic risk to consumers. Nanjing Bestzyme states that the safety of *A. oryzae* 90402 and any derived proteins is further supported by other GRAS conclusions for protein

[3] The subject of GRN 001142 is brazzein produced by *Komagataella phaffii* P-BRZ-013 expressing a gene encoding for brazzein from *Pentadiplandra brazzeana*. We evaluated this notice and responded in a letter dated March 11, 2024, stating that we had no questions at that time regarding the notifiers' GRAS conclusion.

ingredients produced by *A. oryzae* (e.g., GRN 000829).[4]

Based on the totality of information, Nanjing Bestzyme concludes that brazzein preparation is GRAS for its intended use.

Standards of Identity

In the notice, Nanjing Bestzyme states its intention to use brazzein 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 added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity.

Potential Labeling Issues

Under section 403(a) of the Federal Food, Drug, and Cosmetic Act (FD&C Act), a food is misbranded if its labeling is false or misleading in any way. Section 403(r) of the FD&C Act lays out the statutory framework for labeling claims characterizing a nutrient level in a food or the relationship of a nutrient to a disease or health-related condition (also referred to as nutrient content claims and health claims). If products containing brazzein preparation bear any nutrient content or health claims on the label or in labeling, such claims are subject to the applicable requirements and are under the purview of ONFL. OPMAS did not consult with ONFL on this issue or evaluate any information in terms of labeling claims. Questions related to food labeling should be directed to ONFL.

Section 301(ll) of the 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 Nanjing Bestzyme's notice concluding that brazzein 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 brazzein preparation. Accordingly, our response should not be construed to be a statement that foods containing brazzein preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

[4] The subject of GRN 000829 and its supplement is powdered *Aspergillus oryzae* grown with one or more added minerals. We evaluated this notice and responded in letters dated December 9, 2019, and May 18, 2020, respectively, stating that we had no questions at those times regarding the notifier's GRAS conclusion.

Conclusions

Based on the information that Nanjing Bestzyme provided, as well as other information available to FDA, we have no questions at this time regarding Nanjing Bestzyme's conclusion that brazzein preparation is GRAS under its intended conditions of use. This letter is not an affirmation that brazzein 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 001207 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2025.05.23 10:50:09 -04'00'

Susan J. Carlson, Ph.D.
Director
Division of Food Ingredients
Office of Pre-Market Additive Safety
Office of Food Chemical Safety, Dietary Supplements, and Innovation
Human Foods Program

References

1. Renwick, A.G. 2008. The use of a sweetener substitution method to predict dietary exposures for the intense sweetener rebaudioside A. Food and Chemical Toxicology 46:S61–S69.