U.S. FOOD & DRUG ADMINISTRATION
Human Foods Program

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Washington, DC 20001

Re: GRAS Notice No. GRN 001167

Dear Ms. Pelonis:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001167. We received the notice that you submitted on behalf of Perfect Day, Inc. (Perfect Day) on October 23, 2023, and filed it on January 16, 2024. Perfect Day submitted amendments to the notice on June 28, 2024, July 19, 2024, and August 22, 2024, providing additional information about the production organism, manufacturing process, specifications, and safety of the notified substance.

The subject of the notice is brazzein preparation produced by *Komagataella phaffii* strain "BG12"[1] expressing the 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.[2] The notice informs us of Perfect Day's view that this use of brazzein preparation is GRAS through scientific procedures.

Our use of the terms, "brazzein preparation produced by *Komagataella phaffii* strain "BG12" expressing the 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 ONFL regarding the appropriate common or usual name for "brazzein preparation."

[1] FDA notes that *Pichia pastoris* was reclassified as *K. phaffii* as reported in Kurtzman (Ref. 1).

[2] Perfect Day states that brazzein is not intended for use in infant formula or in products under the jurisdiction of the U.S. Department of Agriculture.

Perfect Day provides information about the identity and composition of brazzein preparation. Perfect Day states that brazzein preparation is a white to off-white powder containing ≥90% total protein, of which ≥90% is brazzein, and the remaining protein consists of *K. phaffii* proteins carried over from the fermentation process. The ingredient also contains carbohydrates, ash, moisture, and fat. Perfect Day states that brazzein preparation contains the 53-amino-acid isoform of brazzein naturally present in the fruit of *P. brazzeana*.

Perfect Day states that the production organism, *K. phaffii* strain "BG12", is non-pathogenic and non-toxigenic and is genetically engineered from *K. phaffii* strain "BG10", which is derived from the host strain *K. phaffii* strain NRRL Y-11430. The production organism expresses a gene encoding for brazzein, the constituent responsible for brazzein preparation's sweet-tasting properties. Perfect Day explains that the production organism is constructed via transformation with a single expression cassette, which enables the production of brazzein, and may have multiple copies integrated into the insertion site. Perfect Day confirms that the expression cassette is stably inserted into the genome of the production organism in a single location. Perfect Day states that the production organism does not contain any antibiotic resistance genes or mobile genetic elements.

Perfect Day describes the method of manufacture of brazzein preparation. Brazzein preparation is produced through the controlled fermentation of the production organism, *K. phaffii* strain "BG12". Methanol is used for induction of promoter sequences that promote the expression of the brazzein gene. Brazzein is secreted into the fermentation medium, which is subsequently separated from the biomass by centrifugation. The fermentation supernatant containing brazzein is subjected to microfiltration and ultrafiltration to remove impurities. The solution is then concentrated by ultrafiltration, diafiltration, and resin-based purification. Brazzein preparation is then obtained by concentration and lyophilization. Perfect Day explains that none of the raw materials used during the manufacturing process are, or are derived from, major allergens. Perfect Day states that all raw materials and processing aids used in the manufacturing process are used in accordance with appropriate U.S. regulations or are GRAS for their intended use.

Perfect Day provides specifications for brazzein preparation that include total protein content (≥90% dry weight basis (DWB)), brazzein as percent of the total protein content (≥90% DWB), and limits for moisture (≤10%), ash (≤3%), fat (≤1.5%), carbohydrates (≤2%), ethanol (<10 mg/kg), methanol (<10 mg/kg), heavy metals, including lead (≤0.1 mg/kg), and microorganisms, including *Salmonella* serovars (not detected in 10 g). Perfect Day provides the results from the analyses of three non-consecutive batches to demonstrate that brazzein preparation can be manufactured to meet the stated specifications.

Perfect Day estimates dietary exposure to brazzein preparation based on the relative sweetness intensity of the notified substance and the methodology presented in Renwick, 2008 (Ref. 2). This 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 and an assumption of its substitutional use. Based on the methodology described in Renwick, 2008 and the estimated relative sweetness intensity of brazzein preparation (500 times sweeter than sucrose), Perfect Day estimates the average and upper percentile dietary exposures to brazzein preparation for non-diabetic adults (0.51 and 1.35 mg/kg body weight (bw)/d, respectively), diabetic adults (0.56 and 1.79 mg/kg bw/d, respectively), non-diabetic children (0.85 and 1.98 mg/kg bw/d, respectively) and diabetic children (1.34 and 1.82 mg/kg bw/d, respectively).

Perfect Day discusses publicly available data and information supporting the safety of brazzein preparation and its production organism, *K. phaffii*. Perfect Day describes brazzein as the principal sweetening component of brazzein preparation and notes that it is equivalent to native brazzein protein found in the fruit of the West African *P. brazzeana* plant. Perfect Day 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. Perfect Day describes the biochemical mechanism of brazzein's effects on sweet taste perception and notes that the mechanism is similar to that of other known sweet proteins.

Perfect Day summarizes the results of a comprehensive literature search through May 2024, to identify available safety information relevant to brazzein preparation and does not identify any safety concerns or information that would contradict its GRAS conclusion. Perfect Day provides a summary of a published 90-day subchronic oral toxicity study to support the safety of the intended use of brazzein preparation. Perfect Day describes additional corroborative safety information in the form of unpublished genotoxicity and oral toxicity studies of the subject brazzein preparation. Based on the weight of evidence, including the results of *in silico* sequence-alignment-based approaches, Perfect Day concludes that brazzein and copurified *K. phaffii* proteins do not pose an allergenic or toxigenic risk to consumers. Perfect Day states that the safety of *K. phaffii* and any derived proteins is further supported by the history of safe use of *K. phaffii* in the food industry, including other GRAS conclusions for protein ingredients produced by *K. phaffii* (see GRNs 000204, 000737, 000967, and 001001).[3]

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

Standards of Identity

In the notice, Perfect Day 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

[3] The subjects of GRNs 000204, 000737, 000967, and 001001 are phospholipase C enzyme preparation from *P. pastoris* expressing a heterologous phospholipase C gene, soy leghemoglobin preparation from a strain of *P. pastoris*, soluble egg-white protein produced by *K. phaffii* strain GSD-1209, and myoglobin preparation from a strain of *P. pastoris* expressing the myoglobin gene from *Bos taurus*, respectively. We evaluated these notices and responded in letters dated December 5, 2006, July 23, 2018, September 9, 2021, and December 3, 2021, respectively, stating that we had no questions at that time regarding the notifiers' GRAS conclusions.

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 Perfect Day'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).

Conclusions

Based on the information that Perfect Day provided, as well as other information available to FDA, we have no questions at this time regarding Perfect Day'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 001167 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Mical E. Honigfort -S

Digitally signed by Mical E. Honigfort -S
Date: 2024.10.08 14:46:23 -04'00'

for 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. Kurtzman, C. (2005). Description of *Komagataella phaffii* sp. nov. and the transfer of *Pichia pseudopastoris* to the methylotrophic yeast genus *Komagataella*. International Journal of Systematic and Evolutionary Microbiology, 55, 973-976. doi: 10.1099/ijs.0.63491-0

2. 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.