Jason Ryder, Ph.D.
Oobli, Inc.
202 Cousteau Place, Suite 210
Davis, CA 95618

Re: GRAS Notice No. GRN 001142

Dear Dr. Ryder:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001142. We received Oobli, Inc.'s notice on April 18, 2023, and filed it on August 21, 2023. Oobli Inc. (Oobli) submitted amendments to the notice on December 13, 2023, and February 2, 2024, clarifying information about the intended use, manufacturing process, specifications, technical effect, and safety.

The subject of the notice is brazzein preparation produced by *Komagataella phaffii* P-BRZ-013 expressing the gene encoding for brazzein from *Pentadiplandra brazzeana* (brazzein preparation) for use as a general-purpose sweetener in food at levels determined by current good manufacturing practices.[1] The notice informs us of Oobli's view that this use of brazzein preparation is GRAS through scientific procedures.

Our use of the terms, "brazzein preparation produced by *Komagataella phaffii* P-BRZ-013 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 Center for Food Safety and Applied Nutrition. The Office of Food Additive Safety (OFAS) did not consult with ONFL regarding the appropriate common or usual name for "brazzein preparation."

Oobli provides information about the identity and composition of brazzein preparation. Brazzein preparation is produced using an engineered strain of *K. phaffii* that expresses a gene encoding for brazzein. Oobli states that brazzein is the component of their ingredient that is responsible for its sweet-tasting properties. Oobli further states that brazzein preparation contains primarily protein (approximately 80%), of which

[1] Oobli states that brazzein is not intended for use in foods for which standards of identity exist, infant formula, foods intended for infants, or foods under the jurisdiction of the U.S. Department of Agriculture.

approximately 40% of the total protein is brazzein, and the remaining total protein consists of *K. phaffii* proteins carried over from the fermentation process. The ingredient also contains carbohydrates, ash, moisture, and fat. Oobli states that brazzein preparation that is subject of the notice contains the 53-amino-acid isoform of brazzein (brazzein-53) naturally present in the fruit of *P. brazzeana* Baillon (oubli fruit).

Oobli reports that the production organism, *K. phaffii* P-BRZ-013, is a non-pathogenic, non-toxigenic, and well-characterized yeast with a history of safe use in the food industry. Oobli states that the production organism is derived from *K. phaffii* BG10, which is derived from *K. phaffii* NRRL Y-11430. Oobli states that the production organism is constructed through a series of transformations with different expression constructs to enable the production of brazzein. Oobli states that the production organism does not contain any antibiotic resistance genes or plasmids and is not capable of transferring plasmids or antibiotic resistance to other organisms.

Oobli states that brazzein preparation is manufactured through the controlled fermentation of the production organism. Brazzein is secreted into the fermentation medium and then separated from the biomass. The fermentation supernatant containing brazzein is concentrated, pH-adjusted, and subjected to a sequence of filtration, chromatography, and diafiltration steps. The resulting solution is dried to obtain the final powder form of brazzein preparation. Oobli states that brazzein preparation may contain sodium chloride and sodium acetate that are added as stabilizing agents. Oobli states that none of the raw materials used during the manufacturing process are, or are derived from, major food allergens. Oobli states that all raw materials, processing aids, filtration aids, and pH adjusters used in the production of brazzein preparation are food grade or pharmaceutical grade and are approved for their respective uses in accordance with an appropriate U.S. regulation or are GRAS for their intended use.

Oobli provides specifications for brazzein preparation that include total protein content (>70% w/w), brazzein as percent of the total protein content (>40% w/w), brazzein as percent of total mass (>35% w/w), and limits for moisture (<10% w/w), ash (<10% w/w), fat (<1% w/w), carbohydrates (<15% w/w), heavy metals, including lead (<0.1 mg/kg), and microorganisms, including *Salmonella* serovars (absent in 10 g). Oobli provides the results from the analyses of three non-consecutive batches to demonstrate that brazzein preparation can be manufactured to meet the stated specifications. Oobli states that the shelf life of brazzein preparation is at least 15 months when stored under ambient conditions.

Oobli 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). 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 calculated 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 (330 times sweeter than sucrose), Oobli estimates the average and upper percentile dietary exposures to brazzein

preparation for non-diabetic adults (0.77 and 2.05 mg/kg body weight (bw)/day (d), respectively), diabetic adults (0.85 and 2.72 mg/kg bw/d, respectively), non-diabetic children (1.29 and 3.00 mg/kg bw/d, respectively), and diabetic children (2.04 and 2.75 mg/kg bw/d), respectively.

Oobli discusses publicly available data and information supporting the safety of brazzein preparation and its production organism *K. phaffii*. Oobli 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. We note 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. Oobli describes the biochemical mechanism of brazzein effects on sweet taste perception and notes that the mechanism is similar to that of other known sweet proteins.

Oobli summarizes the results of a comprehensive literature search to identify available safety information relevant to brazzein preparation produced by *K. phaffii*, and Oobli does not identify any safety concerns or information that would contradict its GRAS conclusion. Oobli provides a summary of a published 90-day subchronic oral toxicity study with the subject of this notice to support the safety of the intended use of brazzein preparation. Based on published studies, Oobli concludes that brazzein is non-mutagenic and non-genotoxic. Oobli acknowledges the well characterized metabolic fate of dietary proteins. Based on the weight of evidence, including results of *in silico* and *in vitro* digestibility assessments and published *in silico* sequence-alignment-based approaches to assessing allergenicity, Oobli concludes that brazzein and copurified *K. phaffii* proteins do not pose an allergenic or toxigenic risk to consumers. Oobli states that the safety of *K. phaffii* and any derived proteins is further supported by other GRAS conclusions for protein ingredients produced by *K. phaffii* NRRL Y-11430 (see GRNs 000737, 000967, and 001001, which are incorporated by reference).[2]

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

Standards of Identity

In the notice, Oobli 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

[2] The subjects of GRNs 000737, 000967, and 001001 are soy leghemoglobin preparation from a strain of *Pichia 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 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.

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 in the Center for Food Safety and Applied Nutrition. OFAS 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 Oobli'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 Oobli provided, as well as other information available to FDA, we have no questions at this time regarding Oobli'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 001142 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2024.03.11 17:51:21 -04'00'

Susan J. Carlson, Ph.D.
Director
Division of Food Ingredients
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